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ON THE LIFE-HISTORY OF A FUNGUS PARASITIC ON ANTIRRHINUM MAJUS, WITH SOME REMARKS ON THE GENUS HETEROSPHAERIA.

(With 8 Text-figs.)

By W. Buddin and E. M. Wakefield.

Occurrence and Description of Disease.

In 1917 and 1918 Antirrhinums were received from two different sources showing a leaf disease which appeared to be distinct from anything previously recorded on this host. What was obviously the same disease was noted in 1920 by Miss Cayley and described by her in some detail, though the fungus was not fully identified (1).

Since then the disease has frequently been recorded, and appears to be at present one of the most common and at the same time perhaps the most destructive disease of cultivated Antirrhinums in this country. It does not appear, however, to have been noted outside Great Britain.

A brief recapitulation of the chief characteristics of the disease may be given here.

At suitable temperatures, in about ten days after spraying young and still tender leaves with a spore suspension, there appear the first visible signs of infection in the form of pale green rounded spots, about 5 mm. in diameter. As has been mentioned in a previous note (2), the growth of the mycelium and production of spores of the fungus is active only under somewhat cool conditions, but when once started in a bed the disease spreads very rapidly during spells of cool, moist weather. The leaf spots become more clearly defined owing to the collapse of the affected cells. The subsequent appearance depends to some extent on the variety of Antirrhinum, and also upon weather conditions. The central portion of each spot gradually becomes pale and dries out. Sometimes a definite purplish margin may be developed but in other cases there is no such distinction of colour. Sometimes also the dried areas fall out, leaving holes in the leaf, and the appearance is then like that known as "shot-hole" in plums, etc., or as if the leaves had been eaten by an insect (Fig. 1). Although the leaf phase is always the most conspicuous form of attack, in cases of heavy infestation the green stems also develop lesions which eventually become swollen canker-like areas owing to the production of wound cork beneath the pustules of conidia. When the stems

are infected the flower-stalks may be poor and distorted, and the whole plant has a sickly appearance. Eventually, badly attacked plants are killed outright.

Under suitable moist conditions conidia are produced abundantly on both sides of the leaf, and the numerous acervuli are seen easily with the aid of a pocket lens. Under dry, hot conditions, however, the formation of conidia is temporarily restricted. The acervuli are crowded closely on each spot, are of a very pale pinkish tinge and somewhat waxy or moist in

Fig. 1. Leaves of *Antirrhinum majus* showing spots caused by *Cercosporella Antirrhini*.
Upper row—early stages.
Lower row—showing subsequent "shot-hole" effect.

appearance rather than pulverulent. Vertical sections show that each acervulus arises just beneath a stoma as a minute cushion of closely woven hyaline hyphae. This cushion bears on its outer surface numerous erect, branched conidiophores, which burst through the epidermis and produce at the exterior the curious conidia (Fig. 2, *a–c*). These are at first narrowly obclavate, curved, for a long time non-septate but finally one- to three-septate (Fig. 2, *c*). At length, sometimes only after separation from the conidiophore, the apical portion becomes drawn out into a long fine awl-like point, while after the shedding of the conidia a second but shorter appendage may appear at the base of the spore (Fig. 2, *c*).

Presumably the conidia are washed away in drops of rain or

dew or in the splashes made by watering, and in this way infection is spread. In no case has any other form of fructification been observed on the living plants, and an examination of dead plants left standing in the beds revealed no fungus which could conceivably be regarded as being connected with the Hyphomycete under consideration.

At the time the disease was first received a careful search was made in the literature, but no mention could be found of any fungus occurring on *Antirrhinum* or related genera which was at all suggestive of this species. The fungus was accordingly described as a new species of *Cercosporella, C. Antirrhini* Wakef. (3).

Fig. 2. *Cercosporella Antirrhini*. *a*, Vertical section of acervulus. *b*, Branched conidiophore. *c*, Conidia at various stages. (× 850.)

The reference to *Cercosporella*, as in many cases of Hyphomycetes whose other stages are unknown, was purely a matter of convenience, *Cercosporella* being the nearest "form genus" into which the species would fit. It was recognised at the time, however, that the fungus differed in several respects from a typical *Cercosporella*, and might later have to be removed from the genus.

Temperature Relations of Parasite.

Towards the end of 1923 cultures were started from conidia, and were put on several different media which happened to be available at the time—chiefly Dox's agar, prune agar, and malt extract agar.

At the outset some difficulty was experienced in obtaining abundant growth. Microscopic observations of platings of the conidia on agars revealed the fact that a remarkably high proportion of the conidia failed to germinate, and some rough experiments on the effect of heat on the conidia were therefore carried out.

The conidia germinate slowly and do not lend themselves well to the adoption of the identical technique used by Henderson Smith (4) in his careful study of the effect of heat on the spores of *Botrytis cinerea*. The following method was adopted. Tubes of agar were melted and cooled to the desired temperature in a water bath. After the tubes in each batch had attained the temperature of the bath, equal drops of a heavy suspension of conidia of the fungus were added to each tube, the dilution of conidia in the agar maintained, with shaking, at the particular temperature for one or five minutes, and at the end of the period the contents of the tube were poured quickly into a sterile Petri dish. The dishes were then incubated at 18° C., and after the lapse of an ample period (three weeks) to allow of all viable conidia producing colonies the numbers on the plates were counted.

With malt extract agar as the medium in which the conidia were heated the results shown in Table I were obtained. Very

Table I.

Number of colonies of *Cercosporella Antirrhini* developing on plates of malt extract agar when approximately equal numbers of conidia are heated at the temperatures given, for the times stated, suspended in that medium.

Temperature maintained in water bath	Time of heating	
	1 minute	5 minutes
41° C.	Approx. 10,000	24
43° C.	Approx. 2,500	1
45° C.	5	Nil
47° C.	Nil	Nil

Table II.

Number of colonies of *Cercosporella Antirrhini* developing on plates of prune agar when approximately equal numbers of conidia are heated at the temperatures given, for the times stated, in that medium.

Temperature maintained in water bath	Time of heating		
	Nil (Suspension poured at once)	1 minute	5 minutes
41° C.	Approx. 10,000	—	—
	—	290	Nil
43°, 45° and 47° C.	—	Nil	Nil

similar results were obtained in a duplicate set with malt extract agar, and also with potato gelatine, using different suspensions of conidia. Comparison with the curves of Henderson Smith

indicates that the conidia of *Cercosporella Antirrhini* in malt extract agar and potato gelatine are killed at an appreciably lower temperature (*circa* 5° C.) on the average than those of *Botrytis cinerea* heated in distilled water. The effect of the medium is, however, strikingly shown by the results in Table II, obtained by heating suspensions of the conidia in prune agar (Difco). The conidia were from the same heavy suspension as those used with malt extract agar for the results shown in Table I, and drops of equal size were used for each tube. A duplicate set with prune agar, using a different suspension of conidia, gave very similar results. The figures show that when heated for one minute in the medium the average conidium of *Cercosporella Antirrhini* will withstand a temperature 2–3° C. higher in malt extract agar or potato gelatine than in prune agar. The results also emphasise the advantage of using a gelatine medium when plating out the conidia of an unknown fungus.

The growth of the fungus in pure culture is extremely slow, colonies started from spores not attaining a diameter of 3 cm. until after the lapse of a month at the optimum temperature. This appears to be at approximately 18° C., but growth is almost as good at a low laboratory temperature averaging about 13° C. No growth at all, either from spore inoculations or of colonies of the fungus, takes place at a temperature of 25° C. or higher, although exposure to 25° C. on agar plates for a month does not kill the organism.

It may be noted here that similar temperature relations were found by Vestergren ((5), p. 17) when working with the fungus *Heteropatella cercosperma* (Rostr.) Lind (= *Rhabdospora cercosperma* (Rostr.) Sacc.). In that case temperatures of 25° to 30° C. inhibited conidial formation. The maximum lay between 30° and 35° C. and the optimum at about 20° C. (between 18° and 25° C.). The point will be referred to again in the section on taxonomy.

Growth on Various Media.

Cultures which had been kept for some time were noted during the winter to have produced different growth according to the medium used. On Dox's agar or potato agar the mycelial growth is white and scanty, and closely adpressed to the surface of the medium in a radiating form. Minute pinkish pustules of conidia are developed, on potato agar all over the colony, but on Dox's agar tending to be aggregated towards the centre. No further development was noted on either of these media. On prune agar, however, mycelial growth is rather more vigorous, and in addition to the radially spreading, closely adpressed hyphae tufts of erect filaments consisting of bundles of hyphae

often occur towards the centre of each colony. Conidia are formed abundantly in four days, and at the end of a fortnight there are seen here and there, especially towards the middle of the colony, small black bodies resembling sclerotia.

On malt extract agar the mycelial growth is more compact than on the other media mentioned and conidial pustules develop

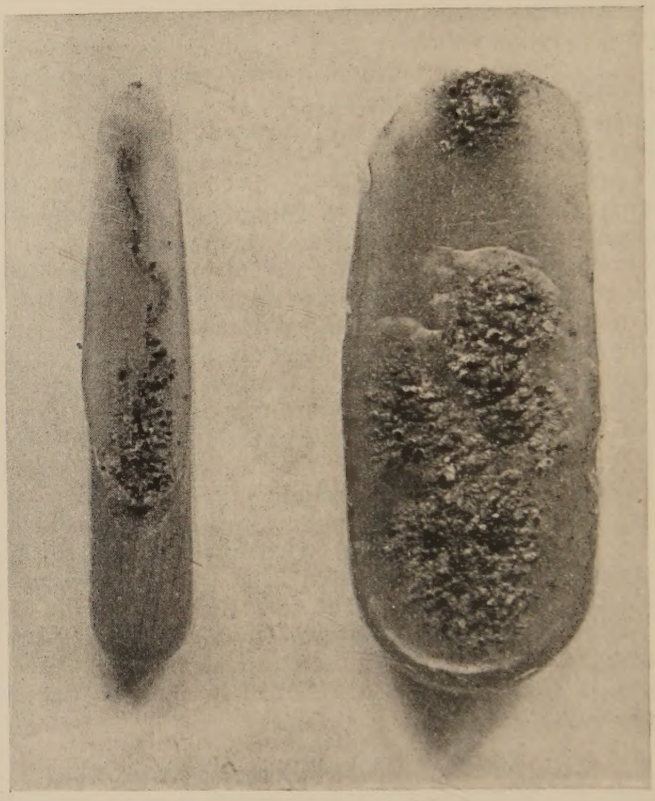

Fig. 3. Two cultures of *Cercosporella Antirrhini* on malt extract agar, showing abundant *Heteropatella* pycnidia (nat. size).

abundantly in the middle of the colony. On this substratum also the sclerotium-like bodies occur, but they are more slowly formed than on prune agar (Fig. 3).

Similar bodies were found later to be produced also on sterilised antirrhinum stems.

As these blackish bodies seemed most likely to throw light on the affinities of the fungus, observations were concentrated

on them. At first they were rounded and compressed in form (more or less "bun"-shaped) and quite solid, black externally but composed within of thick-walled, closely interwoven, colourless hyphae. Later it was noted that some of them had opened out above and become discoid, with a slightly raised, torn margin. The appearance suggested that the bodies represented the perfect form of the fungus and that this was to be sought amongst the Discomycetes. Repeated examination of even the most well-developed discs, however, failed to reveal any trace of asci. Cultures showing them were kept until almost drying up, and were exposed to low temperatures outdoors for 24–48 hours. Further, various media and concentrations of media were tried. If left in the tube in which they were formed, the discoid bodies always remained sterile, the erect filamentous hyphae composing the disc eventually becoming much swollen in parts and obviously abnormal. When transferred to another tube, however, and thus supplied with fresh food material and moisture, the supposed hymenial surface immediately produced an abundant crop of conidia, similar to the original *Cercosporella* conidia.

Over-wintering of Diseased Antirrhinums.

At this point further observation of pure cultures was abandoned, and during the winter of 1924–5 attempts were made to find similar bodies produced in nature. Numerous antirrhinums of several different varieties had been inoculated during the summer and had become badly infected with the disease. Some of these, both at Reading and at Kew, were left standing in the beds; affected leaves and stems of others were placed in empty flower-pots covered with muslin and stood out of doors throughout the winter.

As already mentioned, plants left in the beds gave no result, probably because the stems were not under sufficiently uniform moist conditions. Stems and leaves over-wintered in pots also showed nothing. It was difficult to keep these sufficiently moist and at the same time protected from being eaten by slugs, woodlice, etc., and in London possibly the heavy black fogs of that winter may have had a deleterious effect. At Reading, however, better fortune was met with. In March 1925 numerous decorticated stalks from a large heap of dead Antirrhinums, which during the summer had shown the disease, were found to show an abundance of black Discomycete-like bodies similar in appearance to those formed in pure culture (Fig. 4).

These fructifications proved to be pycnidial only, and although the stems were kept under observation for some months

no ascigerous stage was ever found. From the evidence now obtained, however, it seems certain that the perfect stage, if produced at all, would be a Discomycete. The pycnidial form as found on the over-wintered stems is an undoubted *Heteropatella*, a genus of Excipulaceae, species of which are known

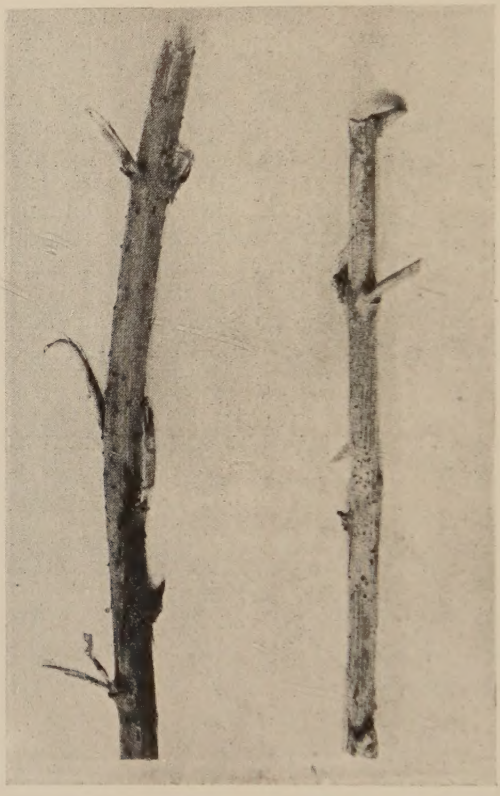

Fig. 4. *Heteropatella* pycnidia developed on over-wintered dead stems of *Antirrhinum* (nat. size).

in other cases to belong to the Discomycete genus *Heterosphaeria*. Apart from the similarity to the bodies seen in cultures, proof of the connection of the *Heteropatella* on the dead stems with the parasitic *Cercosporella* was obtained by inoculation of living Antirrhinums with spore-suspensions from pure cultures of the former, when the characteristic disease spots on the leaves were produced.

Morphology.

(a) *Cercosporella* stage. It has been already stated that the acervuli of the parasitic Hyphomycetous stage develop beneath the epidermis of the leaf or green stem as small hyaline hyphal masses. From these arise erect branched conidiophores which burst through the epidermis in small tufts and produce the conidia externally. The conidiophores are hyaline, branched from near the base, and 2–4 μ in diameter. The conidia arise at the tips of the branches, and are at first non-septate, slightly

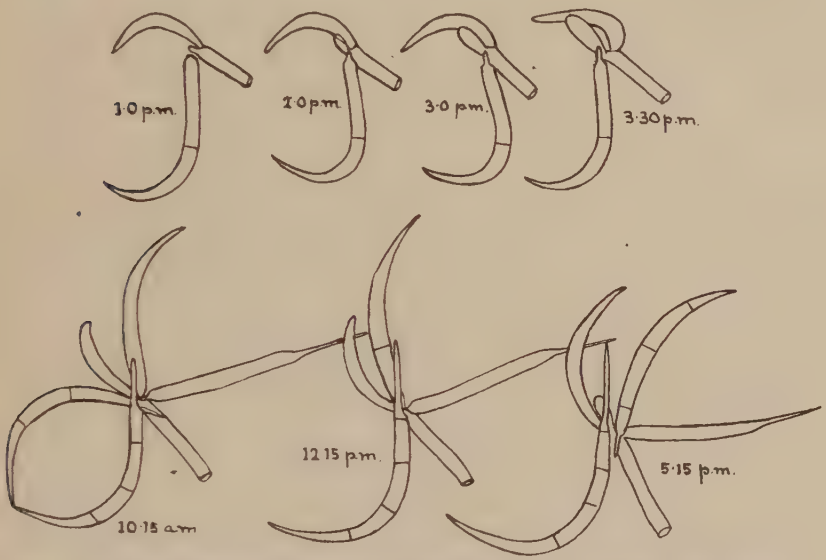

Fig. 5. The production of conidia in a hanging drop. The same conidiophore drawn at intervals on two successive days to show successive development of conidia (× 850).

curved, and narrowed towards the apex but not sharply pointed. The size at this stage is about 25–30 × 2·5–3·5 μ. Gradually the apex becomes more drawn out and at the same time from one to three septa appear, giving the *Cercosporella* appearance which is that usually seen when diseased leaves are examined. After this the conidia fall away from the conidiophores and are no doubt soon washed away. In cultures in hanging drops of sterilised antirrhinum extract the whole development was followed more in detail, and is shown in Fig. 5. The first-formed conidium is pushed to one side by continued growth of the apex of the conidiophore and a second conidium is quickly produced. By the time the primordium of

a third conidium becomes visible between the two previously formed the first conidium has acquired its pointed apex and has begun to show the transverse septa. By continued growth of the third conidium it is gradually pushed more to one side and falls away, remaining (unless disturbed) lying free by the side of the parent conidiophore. Almost immediately after the conidium has become detached it is seen to have a small pedicel-like projection at its base. In the course of a few hours this projection elongates and finally becomes a more or less pointed appendage of about one-third the length of the spore at the time of detachment. Simultaneously the apex of the spore has become drawn out to a long, fine point, with the result that the final form of the spore, prior to germination, is as shown in Figs. 2, *c* and 5. The late development of the basal appendage

Fig. 6. *Heteropatella* stage. *a*, Conidia (× 850). *b*, Vertical section of young pycnidium.

is a point of morphological importance, because the similarly shaped spores of species of *Heteropatella* have by various authors been described as "pedicellate."

The development of the conidium from its first appearance as a small projection at the tip of a conidiophore until it is ready to be detached appears to occupy about seven hours at ordinary room temperatures. After the conidium is shed the development of the basal appendage and the elongated apex may occupy several hours more, but the times have not been exactly noted. The measurements of mature conidia are: body 25–30(–35) × 3·5–4 μ, apical appendage about 20–25 μ, basal appendage up to 10 μ long.

The successive formation of conidia goes on for some time, and in an undisturbed hanging drop fairly large bunches of conidia may be found at the tips of the conidiophores. The slightest motion in the liquid, however, disperses them, and only the two or three conidia as yet immature remain attached.

(b) *Heteropatella* stage. The pycnidial form of the fungus has not been observed on leaves. On dead stems of antirrhinum it arises from the lignified tissue beneath the cortex, and eventually becomes superficial owing to the weathering away of the latter.

At first the pycnidium is a solid, somewhat flattened sclerotium-like body, consisting of a closely-woven mass of hyaline thick-walled hyphae, covered externally by a blackish pseudoparenchymatous cortex. Soon there appears near the outer surface a small flattened cavity, on the floor of which a dense layer of conidiophores is produced. It is now seen that the upper portion of the wall is thinner and different in structure from the basal part, in that the cells show a characteristic radial arrangement (Fig. 6, *b*). Later this lid-like outer wall is torn into segments from the centre outwards, the segments remaining as minute teeth fringing the margin of the flat conidia-bearing disc.

The conidia (Fig. 6, *a*) produced by this *Heteropatella* form of fructification are similar in shape and size to those of the *Cercosporella*, and behave similarly on germination.

Germination of Conidia.

The germination of the conidia, whether those of the *Cercosporella* or of the *Heteropatella*, and the subsequent early stages of development of the colonies, follow in general the course described by Brefeld (6) for the conidia of *Heterosphaeria Patella* and *H. Linariae* and by Vestergren (5) for *Heteropatella cercosperma*. In the media used by the authors, however (distilled water and sterilised antirrhinum extract), the production of secondary conidia immediately, without the formation of mycelium, has not been observed. Germination is always by means of a germ tube.

The cells of the conidium become much swollen, and at first a single germ tube arises laterally from one of the middle cells (Fig. 7, *b*, *d*). Later a second is formed towards the base of the conidium, and in forty-eight hours others may have been produced (Fig. 7, *a*, *c*, *e*). The basal appendage is often visible for a time (Fig. 7, *e*) but is soon obscured owing to the continued development and branching of the hyphae. Meanwhile the first-formed germ tubes have begun to produce conidia. As observed by Brefeld in the case of *Heterosphaeria Patella*, the first conidia are often different in form from the original sickle-shaped spores: they are smaller, aseptate, oblong-elliptical, and straight or only slightly curved, and blunt at each end. After the second or third day the primary conidia are replaced by others which are drawn out at the apex and more distinctly curved (Fig. 8, *a*),

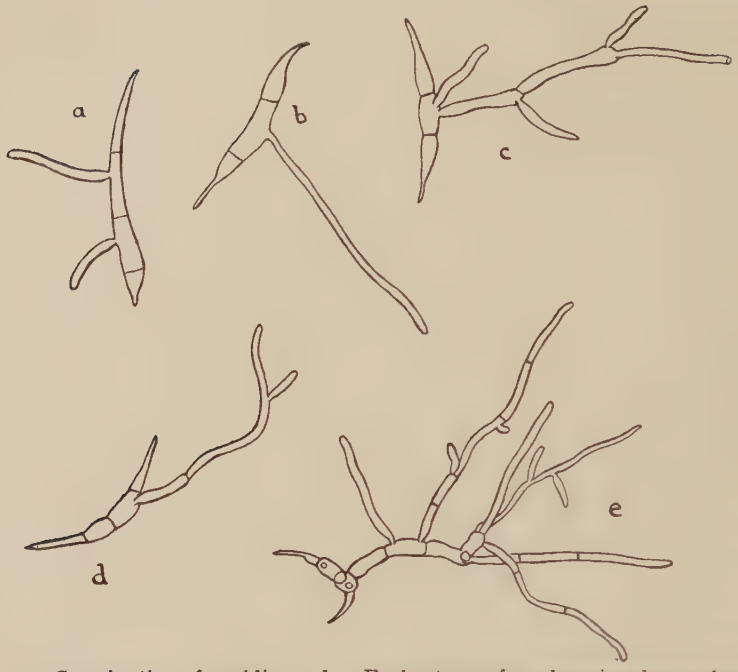

Fig. 7. Germination of conidia. *a, b, c,* Early stages, from hanging drop culture of *Cercosporella* conidia. *d, e,* Later stages, from hanging drop culture of *Heteropatella* conidia.

Fig. 8. Development of mycelium and conidia in hanging drop of sterilised antirrhinum extract. *a,* Normal conidia (early stage). (Culture from a *Cercosporella* spore.) *b,* Showing development of simple cylindrical conidia in a medium which is drying out. (Culture from a *Heteropatella* spore.)

while on the fourth day the normal, septate, sickle-shaped conidia are present in great abundance, and when undisturbed remain hanging together in radiating clusters as already described. There appears to be a tendency for the primary form of conidium to be produced especially when the medium is drying out (Fig. 8, *b*).

Taxonomy.

The discovery of a second "imperfect" fructification in the form of pycnidia belonging to the group Excipulaceae raises some interesting questions with regard to this fungus.

The first obvious comparison is with the so-called "winter conidial stage" which was described by Klebahn (7) in the case of *Entomopeziza Soraueri* and *Pseudopeziza Ribis*. In these two species the summer conidial form, in which the conidia are produced as in the Melanconiales, gives place on over-wintered leaves to a pycnidial fructification. Exactly the same type of conidium is produced, but the conidiophores are enclosed in a wall several layers of cells in thickness, at first closed but later widely open and more or less cup-shaped as in the Excipulaceae.

Klebahn later described a similar state of affairs in *Fabraea Fragariae* (8), where actually the winter conidia occur sometimes side by side with asci in an apothecium-like fruit body, and he compared this with the similar development which had been described by Tulasne (9) for *Heterosphaeria Patella*.

A further interesting point is to be noted in connection with *Pseudopeziza Ribis*. In the form of this species occurring on *Ribes Grossularia*, while the winter pycnidia are very freely produced, Klebahn was unable ever to induce the development of the ascigerous stage.

As already mentioned, the winter pycnidia of the antirrhinum fungus agree in character with the genus *Heteropatella*, species of which are known to be conidial stages of the Discomycete *Heterosphaeria*. Consequently an effort was made to find a perfect stage in this also. Up to the present, however, the search has been without success. Neither in culture nor on the old over-wintered Antirrhinum stems and leaves, even though kept under observation for some months, has there been any indication of the formation of asci.

It is, of course, possible that an ascigerous fructification does occur, and that the right conditions for its production have not been found. The various recorded observations on species of *Heterosphaeria* tend to show that the mature ascophore is formed only under specially favourable conditions. Thus Tulasne mentions that the perfect fruit bodies of *Heterosphaeria Patella* occur on the parts of stems nearest the soil, where the moisture

tends to be more constant. Fries held the view that the ascophore (his form *α alpestre* of *Phacidium Patella*) occurs chiefly in subalpine regions because it requires a constantly damp atmosphere*.

On the other hand, it is not unlikely that we have here a species which, in addition to developing the parasitic habit, has lost or is losing the power of developing asci. Klebahn discusses such a possibility in the case of *Fabraea Fragariae*, of which he says: "Nach den vorliegenden Beobachtungen macht *Fabraea Fragariae* den Eindruck eines Pilzes, der im Begriff steht, die Schlauchfruchtbildung zugunsten der Konidienbildung aufzugeben." Still more to the point is the case of *Gloeosporium Ribis* on *Ribes Grossularia* mentioned above, in which the *Pseudopeziza* stage has not been found, although it is known on other species of *Ribes*.

Comparison of the pycnidial stage of the antirrhinum fungus with described species of *Heteropatella* has shown that morphologically there is little or no distinction. All have conidia which average about 18–25 × 3–4 μ without the appendages, and are from one- to three-septate. There are probably greater differences in the characters of the ascophores, but in the absence of the perfect stage it is difficult to say whether the species in question is new or can be referred to one of the others.

It is interesting to note, however, that there is a species, *Heterosphaeria Linariae* (Rabh.) Rehm, with its conidial stage *Heteropatella lacera* Fuck., which grows on the very closely allied host plant *Linaria vulgaris*. A careful comparison of the Antirrhinum *Heteropatella* with dried material of *H. lacera* has shown that morphologically they are very similar. Unfortunately fresh material of *H. lacera* has not been available, but fresh specimens of *Heterosphaeria Patella* on *Heracleum* were obtained at Tintern in April, 1925, and opportunity was taken to make pure cultures of this species. The growth in culture of *H. Patella* is quite different from that of the antirrhinum fungus, and the latter must therefore be regarded as distinct at least from the common species on Umbelliferous stems.

As there has been some confusion with regard to the taxonomy and nomenclature of the species of *Heterosphaeria*, it may be useful to summarise the facts as far as known.

The genus *Heterosphaeria* was proposed by Greville (10) in 1824, for a plant growing on dead herbaceous stems, chiefly of Umbelliferae, which had been called by Persoon *Sphaeria Patella*. Greville recognised that it was an Ascomycete, though

* This may, however, be due merely to the fact that the species of *Heterosphaeria* in general grow best at low temperatures. See notes on temperature relations above.

not a *Sphaeria*, and described the "thecae," but was unable to find spores. Fries(11) in 1828 had apparently observed, but without understanding, the two stages of the fungus. In his *Elenchus Fungorum*, p. 133, he described two forms of what he called *Phacidium Patella*: the first, *α alpestre*, was described as having an apothecium with asci, while the second *β campestre*, was said to differ in remaining closed, and in never forming mature asci. He described the contents of the supposed closed fruit bodies of his *β campestre* as consisting of "sterile filiform asci."

It was left for Tulasne(12) to interpret the life history correctly, and to show that Fries's form *β campestre* was in reality the pycnidial stage, while his *Phacidium Patella, α alpestre* was the perfect stage of the fungus now known as *Heterosphaeria Patella* Grev.

Previous to Tulasne, Bonorden(13) in 1864 had given a full description of the pycnidial stage, referring it to Greville's *Heterosphaeria Patella*, and suggesting that Greville had been in error in thinking his plant an Ascomycete. Subsequently Hazslinzsky(14) proposed the name *Excipula Bonordeni* for Bonorden's fungus, and this has been recently changed by Lind(15) to *Heteropatella Bonordeni*.

Meanwhile the genus *Heteropatella* had been proposed by Fuckel(16) in 1873, with the one species *H. lacera*, said to grow "on dead stems, chiefly of *Linaria vulgaris*." Fuckel supposed it to be "a Discomycete producing only conidia." The following year Winter(17) discovered asci in the *Linaria* fungus, and gave a description of this ascigerous stage under the name *Heteropatella lacera* (Fuck.) Wint. Rabenhorst later gave the name *Peziza Linariae* (changed by Rehm to *Heterosphaeria Linariae*) to this ascigerous stage, rightly reserving the generic name *Heteropatella* for the Excipulaceous conidial stage to which Fuckel's original description applied.

There seems to be little doubt that the species on *Linaria* and on Umbelliferae are distinct from one another. As indicated by Winter, both apothecia and ascospores are slightly smaller in *Heterosphaeria Linariae* than in *H. Patella*. It is obvious from Fuckel's description and from the specimens on *Linaria* distributed by him in his *Fungi Rhenani* no. 2565, that in the event of the two species being regarded as distinct, as they usually are, the name *Heteropatella lacera* should be applied only to the conidial stage of *Heterosphaeria Linariae* (Rabh.) Rehm. In spite of this however the name seems to have been used loosely even quite recently. Thus von Höhnel (*Ann. Myc.* III (1905), p. 552 and *ibid.* XVI (1918), p. 35) speaks of *Heteropatella lacera* as the "young form of *Heterosphaeria Patella*," and Petrak (*Ann. Myc.* XIX (1921), p. 285) has fallen into the same error.

The best and clearest presentation of the common European species is that given by Lind in his *Danish Fungi* (15), where he has the following pairs of forms:

Ascigerous	Conidial
Heterosphaeria Patella Grev.	*Heteropatella Bonordeni* (Hazsl.) Lind.
Heterosphaeria Linariae (Rabh.) Rehm.	*Heteropatella lacera* Fuck.
Heterosphaeria Patella v. *alpestris* Fr.	*Heteropatella cercosperma* (Rostr.) Lind.

With regard to the third species, the conidial stage is that described by Vestergren in detail under the name *Rhabdospora cercosperma* (Rostr.) Sacc. It is obvious from the description and figures that the species is a *Heteropatella*. Vestergren recorded it as growing on numerous plants belonging to very different Natural Orders, in alpine and arctic regions, and for this geographical reason Lind has referred it to Fries's var. *alpestre* of *Heterosphaeria Patella*. It seems probable, however, that the name includes several species, and in view of the fact that Vestergren found temperature relations which agree with those of our Antirrhinum parasite, and may therefore be common to the whole group, it does not appear to be wise to take up the name *alpestris* with no further distinction. Obviously more exact work, with modern methods, is required as to the host relations and cultural characters of the Heterosphaerias.

In conclusion, reference must be made to the parasitic Hyphomycetous stage, *Cercosporella Antirrhini* Wakef., which was the starting point of the present investigation. In none of the known species of *Heterosphaeria* has such a stage been recorded. There is however a fungus which from the published descriptions is suggestively similar. This is a species on *Bupleurum* which was referred to *Heteropatella hendersonioides* Fautr. et Lamb. by Diedicke (18). Fautrey and Lambotte described their fungus on *Bupleurum falcatum* as having a pycnidium, and the spores as having three basal setae, Diedicke found his species on *Bupleurum longifolium*, and rightly or wrongly referred it to the same name. According to Diedicke, however, the structure of his plant is that of the Melanconiales, and not of a *Heteropatella*; that is, it has no pycnidial wall, but produces conidia in a layer covered only by the epidermis. Further, he was never able to see more than one basal appendage.

As every systematist knows, the distinction between the Melanconiales and the Hyphomycetes in certain species is difficult to draw, being a question of degree of development of the sporiferous layer. Diedicke's fungus, which he referred tentatively to *Pestalozzina* (19), is distinctly reminiscent of *Cercosporella Antirrhini*; it may possibly also have been para-

sitic, as it is said to occur "on extensive dry patches on leaves and stems."

It seems possible that the loss of a definite limiting pycnidial wall may be a new development in the life history of *Heterosphaeria* correlated with the adoption of a parasitic mode of life. One may perhaps go further and suggest that the antirrhinum parasite has arisen as an adaptation—a mutation even—from the species *Heterosphaeria Linariae* which occurs on the very closely allied host plant *Linaria vulgaris*, and that with the adoption of the parasitic mode of life it has lost the power to form asci and survives from season to season by means of the winter pycnidia.

Such a supposition would explain why an organism causing such conspicuous injury should yet apparently not have been described until so recently as 1918. It may be mentioned that one attempt has been made to inoculate *Linaria* with the antirrhinum fungus, but without result. It is hoped to do some further work in this direction.

Summary.

The paper records observations on the life history of *Cercosporella Antirrhini* Wakef., described for the first time in 1918 as a parasite of cultivated Antirrhinums.

In pure culture on certain media blackish apothecium-like bodies are produced, which however remain sterile unless transferred to fresh tubes, when they produce conidia.

Similar bodies were found on over-wintered dead stems of Antirrhinums which had shown the disease in the previous summer. These bodies were definitely pycnidial fructifications and were recognised as belonging to the genus *Heteropatella* Fuck.

The connection with the *Cercosporella* was proved by inoculation of living Antirrhinum leaves with conidia from pure culture of the *Heteropatella*, when the characteristic leaf-spots were produced.

No ascigerous stage has as yet been found.

Relationship with *Heteropatella* is further confirmed by the temperature relations of the fungus, which agree with those found by Vestergren for a species identified as *Rhabdospora cercosperma* (Rostr.) Sacc. (= *Heteropatella cercosperma* (Rostr.) Lind).

The optimum temperature lies at approximately 18° C., and no growth takes place at temperatures of 25° C. or higher. The conidia are killed by heating at 41° C. for five minutes in malt extract agar, and after one minute in prune agar.

A discussion of the taxonomy of the genera *Heteropatella* and

its ascigerous stage *Heterosphaeria* is appended, and it is suggested that the *Cercosporella* may be a new development in the life history of *Heterosphaeria* correlated with the adoption of the parasitic habit. The antirrhinum fungus may have arisen as a saltation from *Heterosphaeria Linariae*, which occurs on dead stems of the closely related host plant *Linaria vulgaris*.

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SUPPLEMENTARY NOTE.

By W. Buddin and E. M. Wakefield.

Since the foregoing paper was submitted for publication the authors have obtained living material of *Heteropatella lacera* Fuck. through the kindness of Mr A. S. Thomas, B.Sc., who collected it on old stems of *Linaria vulgaris* near Odiham, Hants, in the early spring of this year. Pure cultures of this species have been made, and compared as to growth characters and pathogenicity with those of the Antirrhinum parasite.

Morphologically there is very little distinction between the pycnidia of the two fungi. Those of *H. lacera* are larger ($\frac{1}{2}$ to

1 mm. in diameter), and more decidedly black from the beginning than the small, brownish to black bodies which are found on old stems of *Antirrhinum*. The conidia of *H. lacera* have also perhaps slightly longer appendages, and are for the most part more strongly curved than those of the Antirrhinum fungus, but these differences are slight.

Cultural characters have given more definite information. Germination of the conidia in both cases is by means of one or more germ-tubes. In distilled water there is little difference, but with further growth on solid media, such as Dox's agar, distinct differences in development are to be observed between the two forms. In *H. lacera* from *Linaria* the short, cylindrical, budded-off conidia which were figured by Brefeld are formed very abundantly, so much so that a yeast-like mass is soon developed on the surface of the medium. The characteristic, mature, sickle-shaped conidia appear only at a rather late stage, and in relatively small numbers. In cultures of the Antirrhinum fungus, on the other hand, the short conidia are not formed in any great abundance except when the medium is drying out, whereas the sickle-shaped conidia appear within a few days and are the predominant form. Moreover there is, as a rule, more aerial mycelium developed in the case of the Antirrhinum fungus, so that cultures of the two fungi have from the first slightly different facies. In cultures of *H. lacera*, again, the blackish pycnidia begin to appear at an early stage, and in abundance, even on Dox's agar. As already described, those of *Cercosporella Antirrhini* appear freely only in older cultures on organic media such as prune or malt agars.

The pycnidia of *H. lacera* as formed in culture are black, and when looked at with a lens appear slightly hairy externally. They remain for a long time closed. Those of *Cercosporella Antirrhini* on the other hand are at first brownish in colour, quite smooth externally, and soon develop the open, discoid form. In neither are the marginal laciniae so conspicuous in culture as in nature.

Confirmation of the differences between the two fungi was obtained by means of inoculation experiments. Young plants of *Antirrhinum* were sprayed with spore-suspensions prepared from pure cultures of each form. Those sprayed with conidia from cultures of the *Heteropatella* from Antirrhinums developed lesions on leaves and stems in about 14 days. Later the leaves showed the characteristic shot-hole effect, and many of the young green shoots were completely killed. Plants sprayed at the same time with conidia from pure cultures of the *Linaria* fungus remained perfectly healthy. The experiment was repeated with similar results. It may also be mentioned that an

attempt to inoculate *Linaria* in 1925 with conidia from the Antirrhinum form gave negative results.

There is therefore no doubt that the fungus causing shot-hole disease of Antirrhinums is distinct from the closely allied form on *Linaria vulgaris*. It differs in parasitism, and further differs from all other known species of *Heteropatella* in possessing a summer conidial stage which is morphologically a Hyphomycete. While this latter is obviously not really co-generic with other more typical Cercosporellas, there is at present no other form genus in which it could be better placed. In view of the occurrence of a definite pycnidial stage having the same type of spore, it will be preferable to adopt the name of the pycnidial stage than to create a new genus for the summer hyphomycetous stage. Since the pycnidial stage has been shown to be biologically and to some extent morphologically distinct from *Heteropatella lacera*, it should receive a new specific name, and may be called *Heteropatella Antirrhini* Buddin et Wakef.

In spite of further search no trace of an ascigerous stage has yet been found.

Heteropatella Antirrhini Buddin et Wakef. sp. nov.

Pycnidia in ligno decorticato plantae nutricantis superficialia, sparsa, circa 500–600 μ diametro, e brunneo nigrescentia, globoso-depressa, primo clausa, deinde in lacinias irregulares dehiscentia. Substantia interna coriacea, albida, ex hyphis crasse tunicatis densissimeque intertextis composita. Discus conidiophorus planus, carnosus, pallidus. Conidiophora dense verticaliter disposita, filiformia, ramosa. Conidia falcata, utrinque attenuata, hyalina, 2–3-septata, 25–30(–35) × 3–4 μ, apice appendiculo filiformi, 20–25 μ longo praedita, basi propter appendiculum breviorem quasi pedicellata, in globulos pallide roseos emergentia.

Hab. In caulibus emortuis decorticatis *Antirrhini majoris*. Adest status hyphomycetalis parasiticus, *Cercosporella Antirrhini* Wakef. foliis praecipue valde noxius.

CRYPTOTHECIACEAE.

A FAMILY OF PRIMITIVE LICHENS.

(With Plate VII.)

By A. Lorrain Smith.

Some years ago, as part of my study of British lichens, I had the privilege of looking over the late Dr Stirton's large collection of British and foreign specimens. Cordial thanks are due to the authorities in Glasgow, to whom the Stirton herbarium had been consigned, for giving me the opportunity to examine, and thus include in the *Monograph of the British Lichens*, the plants, new or old, determined by him. Dr Stirton was an indefatigable collector and worker with a wide knowledge of lichens: he has added several species to the British flora.

The greater number of specimens in his collection were, however, exotic, sent to him by friends from many lands—Canada, South and West Africa, New Zealand, Australia, India and elsewhere. Many of the plants had been determined, rightly or wrongly, by Stirton and include many new species. The greater number, however, enclosed in neat packets, bear a label inscribed only with the date, the name of the collector, the locality and, as a rule, the generic name. Pressure of other work prevented any study of the unnamed specimens, but records were kept of those with definite determination.

Among the more interesting were the plants labelled *Cryptothecia*, a puzzle to all lichenologists since the publication of a species, *Cryptothecia subnidulans*, by Stirton in *Proc. Phil. Soc. Glasgow*, x (1877), p. 164, as follows:

"*Cryptothecia subnidulans* sp. nov.

"Thallus albidus vel pallidus vel passim pallide cinereo-glaucescens, nonnihil farinaceus, molliusculus saepe chrysogonidicus, ambitu radiatim byssino-fibrillosus, bene evolutus (iodo sordide coerulescens, sed C-); apothecia nulla (propria sic dicta) visibilia, solum apicibus thecarum thallo obtectis vel nudis, protrudentibus vel prominulis (C. erythrinosis); thecae late ovatae monosporae rarissime 2-sporae in fibrillis anastomosantibus inclusae, sporae incolores oblongae interdum curvulae murali-divisae, episporio tenuisculo, ·05–·075 × ·02–·032 mm., iodo flavescentes. Iodo gel. hym. coerulescens vel (in speciminibus africanis) obsolete coerulescens vel haud tincta.

"Ad cortices arborum in montibus Nilgherrensibus (Dr. Watt) et prope Victoriam in Africa tropicali (Thomson).

"This lichen is allied to *Arthonia aleurodes* (Nyl.) and *A. subsimillima*.

"The thecae are found pretty equally distributed throughout the thallus, and not congregated in groups so as to constitute true apothecia; accordingly, I have separated this and several others from the genus Arthonia."

The genus, as such, was placed on record by A. Zahlbruckner in Engler and Prantl, *Nat. Pflanzenf.* 1, 1* (1903), p. 92, as "imperfectly known and of obscure position": his statement stands as the first publication of the genus.

After due consideration I have come to the conclusion that *Cryptothecia* is the representative of an entirely new family of lichens—primitive in the formation of the fructification, though advanced in spore characters, and, among lichens, most nearly associated with the Pyrenolichen families—Thelocarpaceae and Mycoporaceae. The nearest fungal allies are apparently the Myriangiales* which *Cryptothecia* resembles in the solitary embedded asci without paraphyses. There is also undoubtedly a close affinity with the Plectascales, especially with Gymnoascaceae, in which family a similar loose peridium surrounds a fruiting body, differing in the numerous asci but also without paraphyses. The characters of these two groups in the case of the fungi suggest a relationship or a common ancestor. The difficulty of deciding the systematic position of the Myriangiales was appreciated by G. Arnaud who discusses the problem in his work on Les Astérinées (*Ann. Sci. Nat.* Sér. 10, VII (1925), p. 653. He decided finally that the Myriangiales were derived evidently from some type of primitive Ascomycetes: "One could," he states, "consider them as Plectascineae (for example Gymnoascaceae) in which the felted tissue which surrounds the asci would have become a compact tissue."

It was evidently the presence of the loosely interwoven peridium that led Stirton to suggest affinity with the Arthoniaceae in which there is a dense epithecium of branching paraphyses. The ascus of *Cryptothecia* with its peridium is a separate fruiting body each isolated in its covering of hyphae—though occasionally in groups—and deeply embedded during the whole development in the thalline tissue. It emerges readily on pressure when mature.

CRYPTOTHECIACEAE.

Thallus heteromerus, crustaceus, epiphloeodes, effusus interdum determinatus, non corticatus, hyphis medullaribus substrato adfixus. Gonidia palmellacea, vel stratum gonidiale

* It is interesting to note that for a long time the genus *Myriangium* was classified as a lichen, and placed as the solitary member of a family Myriangiacei. (See Crombie, Monogr. Brit. Lich. 1, pp. 12 and 15, 1894.)

vel glomerulos discretos formantia. Fructificatio pyrenocarpea, in thallo immersa; perithecia solitaria, dispersa vel approximata; peridium ex hyphis ramosis, contextis, tenuibus formatum, ascum singulum (vel binum?) continens; paraphyses nullae; sporae 1–8nae murali-divisae vel septatae. Pycnidia non visa.

Family CRYPTOTHECIACEAE.

Thallus crustaceous, epiphloeodal, superficial, or entangled with the outer cells of the bark without a cortex, rather thin, and compact. Algal cells, Palmellaceae (?) bright green, small, subglobose, about 7–10 μ in diameter or subellipsoid. Reproductive body a perithecium containing one ascus, solitary, sparse or sometimes in groups, each with a peridium of colourless or brownish loosely-wefted hyphae, immersed in the thallus beneath the gonidial zone; paraphyses not formed; spores 1–8 in the ascus, colourless, muriform or septate.

The thallus, a sterile-seeming crust, recalls that of *Crocynia*, but is of denser texture. Stirton has described the fruit bodies as finally protruding from the thallus, an observation I have failed to verify, but the specimens, with one exception, were collected about fifty years ago and in several instances only empty asci, or stray spores have been found, though marked by Stirton as fertile. Stirton has recorded two asci in the perithecium: I have not been able to confirm that. The perithecia may be widely spaced or developed in close proximity.

The gonidia as far as seen are bright green, no chrysogonidia (*Trentepohlia*) as recorded by Stirton have been observed.

The lichen may evidently spread to two or three inches or more and in some instances it covers other crustaceous forms thus indicating a quick vigorous development; it grows on the uneven surface of the bark or round twigs, and occurs sometimes in quite small patches. The thallus is mostly very light-coloured sometimes with greenish patches where the gonidia have come near to the surface and sometimes darker at the edges. It is frequently covered with a fine furfur of minute cells budded off from the slender hyphae and the medulla may also be inspersed with similar cells; occasionally the ends of the hyphae project from the cortex. When water is applied it runs off in most of the specimens leaving the non-gelatinous surface dry.

It is generally impossible to determine before making sections the position of the fruit bodies in any specimen, though they tend to develop in the thicker portions, or in small wart-like nodules and pustules when these are present.

The specimens from India were collected by Sir G. Watt; those from West Africa by Mr G. Thomson.

Two genera, differing in spore characters, are represented among Stirton's specimens:

Spores muriform	.	1. *Cryptothecia.*
Spores septate	.	2. *Stirtonia.*

1. ***Cryptothecia*** Stirton apud A. Zahlbr. *loc. cit.*

Crusta effusa, sat tenuis, subdeterminata, non corticata. Gonidia minuta, in stratum continuum vel glomeratum disposita. Perithecia infra stratum gonidiale immersa; asci solitarii; sporae 1–8nae, incolores, murali-divisae.

In the genus *Cryptothecia* the thallus is spreading, smooth or finely furfuraceous or slightly uneven with small nodules or wrinkles, whitish or greyish, effuse or sometimes white fimbriate at the margins and determinate, about 100 μ to 250 μ thick; upper cortical tissue intricate, composed of slender closely-wefted hyphae, and about 30 μ from the surface to the gonidial zone; gonidia in an irregular loose layer varying in depth to about 40 μ and subcontinuous or occasionally in patches, the medulla of rather densely entangled hyphae similar to those of the cortical layer adhering closely to the substratum and entangled with the cells of the outer bark. Perithecia ovoid, pyriform or almost spherical, entirely immersed below the gonidial zone, the peridium of wefted hyphae about 5–10 μ thick; ascus almost as large as the perithecium, generally thick walled over the apex; spores 1–8 in the ascus, colourless, broadly oblong or ovoid, muriform.

Ascus 1-spored.

1. ***C. subnidulans*** (Stirton, *loc. cit.* descr. generica nulla) A.L.Sm.

Thallus cinereus vel albidus effusus, uniformis, laevigatus vel verrucis minutis obsitus, tenuis, usque 200 μ altus, margine fimbriato albido cinctus (K–, CaCl–, vel erythrinosa). Perithecia subpyriformia, usque 100 μ long. 55 μ lat.; sporae 1-nae, hyalinae, multi-septatae et murali-divisae 50–60 μ long., 25–30 μ cr., cellulis parvis, numerosis.

Distr. West Africa (Victoria, Cameroons); South-east Africa (Natal); India, Bengal (Chinsurah), Madras (Nilghirri Hills). Pl. VII, figs. 1, 2, 3.

The thallus is smooth or minutely nodulose, whitish or light cinereous-grey, subdeterminate and slightly white-fimbriate at the margins, rather widely spreading, usually about 150 μ in depth (K –, CaCl + rose-red ov. –). Perithecia developed beneath the gonidia, easily emerging from the peridium when mature, the peridium about 5–10 μ thick, becoming brownish; the single ascus pyriform, thick walled (about 10 μ thick), very

variable in size from 60 to 100 μ long and from 45 to 55 μ wide; the spores, one in the ascus, up to 14 or more septate and muriform, variable in form but generally broadly oblong, sometimes curved, varying also in size from 50 to 65 μ long, 25 to 30 μ thick.

On testing with potash there is sometimes a faint yellow reaction which soon disappears. The rose-red colour on the application of calcium hypochlorite is also somewhat impermanent or not present.

The specimen recorded from South-east Africa differs slightly though not specifically; it was sent to me some years ago by Professor Van der Byl of Berea, Natal. It grew on the bark of *Albizzia fastigiata*; the thallus is lighter in colour and thicker than in the specimens from more tropical lands, reaching a depth of ½ mm. or even more. The tissue is somewhat loose, the hyphae appreciably stouter and the gonidia more scattered, 8–10 μ diam. and not easy to determine, though evidently Palmellaceae. These characters possibly indicate a moister habitat. The reaction with CaCl is + yellow then reddish, but slow to appear, and impermanent; the colour was obtained by using Magnusson's method of introducing a grain of bleaching powder into the water-mount. The periderm is rather thin, the ascus about 70 μ × 45 μ and the spore variable in size, 45–60 μ long, 15–22 μ thick; and irregularly muriform. Periderm I+ scarcely blue, ascus and spore red.

Ascus up to 8-spored.

2. ***C. Stirtonii*** A.L.Sm.

Thallus crustaceus, albidus vel cinereus, effusus subdeterminatus, margine interdum byssino-fibrillosus. Perithecia numerosa, peridio fibrilloso inclusa. Asci solitarii; sporae 8-nae, oblongae hyalinae, ca. 10-septatae et murali-divisae, 70–100 μ long., 35–50 μ lat., cellulis parvis numerosis. Pl. VII, fig. 4.

Distr. West Africa (Cameroons); Burmah (Rangoon); India (Tongloo, Himalayas 10,000 ft.), and Bengal (Chinsurah).

The thallus of *C. Stirtonii* scarcely differs from that of *C. subnidulans*: it is whitish, grey or greenish-cinereous, effuse or subdeterminate and thinly white-fimbriate at the margins, smooth or with occasional pustules (K −; CaCl −, I + blue in part). Perithecia situated beneath the gonidial zone, subpyriform or almost spherical, varying in size, but rather large: the peridium becoming brownish about 10 μ thick; ascus with a wall 10 μ thick or more and varying in size up to 130 μ long and 80 μ wide, or, if spherical, about 120 μ diam.; spores 8 in the ascus, large and broadly oblong, sometimes curved, but straight when

properly mature, also varying in size from 70 μ long and 35 μ thick to 100 μ long and 50 μ thick, about 10 or more septate and muriform, with 6 or more longitudinal divisions, the cells small and numerous.

The different specimens had been labelled as *Cryptothecia* and in some instances spore characters were added. I have named it in honour of Dr Stirton

3. ***C. subtecta*** (Stirton MS.) A.L.Sm.

Thallus albidus, cinereus, vel fusco-cinereus, laevigatus vel leviter purpuraceus, subdeterminatus (K – vel flavescens, CaCl –). Perithecia immersa, vel sparsa vel crebra, peridio hyphis fuscescentibus composito, ovoidea 70 ad 100 μ long. 50–70 μ lat.; asci peritheciis subaequantes, membrana sat tenuis; sporae 8-nae, oblongae, 14-septatae et irregulariter murali-divisae, usque 50–40 μ long. 15–20 μ cr., cellulis parvis, numerosis. Pl. VII, fig. 5.

Distr. India, Bengal (Chinsurah) and Assam.

The thallus of *C. subtecta* is about 150 to 200 μ in depth; the algal cells about 7 μ diam. at times almost reaching the surface. Perithecia solitary or rather closely grouped, pyriform-ovoid from 70–100 μ long, 50–70 μ in width, the peridium brown in thick section; asci almost filling the perithecium, with a thin membrane about 5 μ in width; spores broadly oblong, irregularly muriform; about 14-septate and 5 to 6 longitudinal divisions, 30–40 μ long, 15–20 μ thick.

The specimens were labelled by Stirton as *C. subtecta*; the perithecia are completely hidden in the thallus without any indication of their presence. It differs from *C. Stirtonii* in form and size of perithecia, spores, etc. There is occasionally a slight tinge of yellow on the application of potash; the reaction with iodine is variable, the perithecia and parts of the thallus are occasionally tinged blue.

4. ***C. conferta*** A.L.Sm.

Thallus tenuis, albido-cinereus vel virescens, laevigatus vel leviter verruculosus, subdeterminatus et margine fimbriatus (K – , CaCl –). Perithecia normaliter ad basim inter sese conferta sed distincta, ovoideo-elongata usque 80 μ long. 45 μ lat. Peridium hyphis peridiis fuscescentibus compositum; asci membrana tenuis sed apice crassior usque ad 10 μ lat., sporae 8-nae, ovoideae, 6 9-septatae et irregulariter murali-divisae, ca.30 μ long. 17 μ cr., cellulis parvis, numerosis. Pl. VII, fig. 6.

Distr. India, Assam.

The thallus is thin, about 140 μ thick, greenish or greyish white, smooth or dotted with minute nodules, subdeterminate and indistinctly fimbriate at the margins. Algal cells are 7–10 μ diam.

in a gonidial zone or in dense groups near the surface sometimes giving a greenish appearance to the thallus. Perithecia with a brownish peridium, elongate-ovoid, about 85 μ long, 40 μ wide occurring in close groups attached at the base; the ascus with a narrow wall widening over the tip to about 10 μ; spores 8 in the ascus, elongate-ovate, with a clear gelatinous wall, irregularly muriform, 6–9-septate with 2–4 longitudinal divisions 25–30 μ long, 10–17 μ thick. *C. conferta* marks a distinct association of the perithecia, a character somewhat suggesting the pseudo-stromata of Mycoporaceae. It differs from other species in the closely approximate perithecia and in the smaller spores.

2. *Stirtonia* A.L.Sm. gen.nov.

Thallus crustaceus, effusus. Algae Palmellaceae minutae. Perithecia in thallo immersa, peridio e hyphis contextis composito, ascum singulum continens; sporae 8-nae, hyalinae, pluri-septatae.

The thallus does not materially differ from that of *Cryptothecia*. I have pleasure in naming the genus after Dr Stirton.

1. ***St. obvallata*** A.L.Sm.

Thallus sat parvus, cinereo-albidus, fibrillae marginis paulum distinctae (K −, CaCl −, I + caerulescens). Stratum gonidiale non bene distinctum, gonidia parva ca. 7 μ diam. Perithecia immersa; asci fere ovoidei, 90–110 μ long., 55–70 μ lat. Sporae oblongae, rectae vel leviter curvulae, membranis cinctae, 6–8-septatae, 50–90 μ long. 20–30 μ cr. Pl. VII, fig. 7.

Distr. India, Bengal (Chinsurah).

Stirtonia obvallata is distinguished by the thin thallus with a very distinct reaction with iodine; the gonidial layer is also a thin layer of small scattered algae about 7 μ diam. and delicate intricate hyphae. The perithecia are deeply immersed, the peridium also becoming blue with iodine; the asci somewhat ovoid 90–110 μ long, 55–70 μ wide; the spores broadly oblong sometimes slightly curved with a clear epispore, very distinctly and mostly 7-septate, 60–90 μ long, 20–30 μ thick.

The species was labelled by Stirton as *Cryptothecia obvallata* but not published. It was collected by D. G. Watt.

2. ***St. dubia*** A.L.Sm.

Thallus effusus, pertenuis, albido-cinereus, leviter furfuraceus (K −, CaCl −, I + caerulescens). Gonidia minuta, ca. 5 μ diam. Perithecia immersa, peridio e hyphis contextis composito; ascus ovoideus, usque 55 μ long. 30 μ lat.; sporae hyalinae fusiformes, elongatae, rectae, normaliter 7-septatae, 25–30 μ long. 7 μ cr., I + vinose rubens. Pl. VII, fig. 8.

Distr. India, Bengal (Chinsurah).

There is only one specimen of this lichen which is growing on wood and is bordered by a dark line possibly due to an adjoining species. The thallus is extremely thin and the different tissue layers difficult to determine; there is a distinct blue reaction with iodine, especially in the neighbourhood of the perithecia. The algal cells are small and scanty. The peridium enclosing the perithecium is rather thin; the spores are distinctly septate without longitudinal divisions, elongate-fusiform 25–30 μ × 7 μ with the middle cells slightly larger. It is possible that they might be immature and ultimately become more or less muriform but the form of the spores as well as of the septation agrees with *Stirtonia*.

I am indebted to Mr E. H. Ellis for the photomicrographs and assistance with the drawings.

EXPLANATION OF PLATE VII.

Fig. 1. Section through thallus of *Cryptothecia subnidulans* showing (×45): *a*, bark of tree; *b*, immersed perithecia; *c*, position of gonidial layer; *d*, cortex.
Fig. 2. *C. subnidulans*: ruptured peridium and ascus with spores. ×180.
Fig. 3. *C. subnidulans*: spore. ×440.
Fig. 4. *C. Stirtonii*: ruptured peridium and ascus with spores. ×125.
Fig. 5. *C. subtecta*: spore. ×440.
Fig. 6. *C. conferta*: ascus with spores. ×440.
Fig. 7. *Stirtonia obvallata*: ascus with spores. ×440.
Fig. 8. *S. dubia*: ascus with spores. ×440.

THE GENUS LIGNIERA MAIRE & TISON.

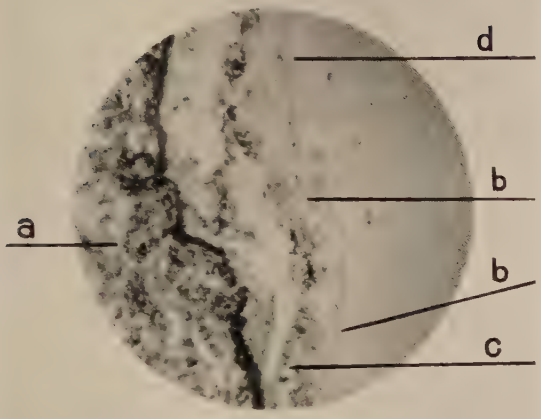
(With Plates VIII and IX.)

By W. R. Ivimey Cook, B.Sc.

Introduction.

In the genus *Ligniera* have been placed those Plasmodiophoraceous fungi which cause no, or practically no hypertrophy of the host tissues. They occur solely in the roots of Phanerogams.

In 1910 Blomfield and Schwartz (1) described and figured the life history and cytology of *Sorosphaera Veronicae* which caused hypertrophy of the stems of *Veronica Chamaedrys*. Later in the same year and again in 1911 Schwartz (20, 21), described two further species, *Sorosphaera Junci* and *S. graminis* occurring in the roots of *Juncus articulatus* and *Poa annua* respectively. Maire and Tison in 1911 (11, 12), constituted the genus *Ligniera* to include two species which they had themselves discovered, *L. verrucosa* in the roots of *Veronica arvensis*, and *L. radicalis* in those of *Callitriche stagnalis*. They also transferred *Sorosphaera Junci* to their new genus *Ligniera* on the ground that unlike *S. Veronicae* it caused no hypertrophy of the host tissues. The following year Winge (23), reviewing the Plasmodiophorales, included in the genus *Ligniera* the three



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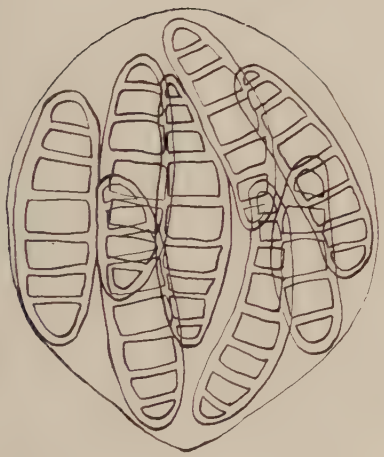

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CRYPTOTHECIACEAE

species already described and also added *Sorosphaera graminis* the life history of which had been published before Maire and Tison's paper had been received in this country. In 1914 Schwartz (22) adopted these alterations and briefly described three more species of *Ligniera* from the same locality where he had obtained *L. Junci* and *L. graminis*, and named them after their host plants *L. Bellidis*, *L. Menthae* and *L. Alismatis*.

In 1925 Fron and Gaillat (6) described from France a species of *Ligniera* occurring in the root hairs of *Poa annua*, which they called *L. pilorum*. They separated *L. pilorum* from *L. radicalis* on the ground that their species caused some hypertrophy of the root hairs. They did not, however, cite Schwartz's paper on *Sorosphaera graminis* in which the fungus is recorded as attacking the roots of *Poa annua*.

In the summer of 1924 material of *Ranunculus circinatus* infected with a species of *Ligniera* was given to me by Dr E. J. Schwartz. Unfortunately the roots were not sufficiently well preserved to enable me to make out any cytological detail. This material had been collected from the same locality as the other species of *Ligniera* which he had previously described. In the autumn of 1924 a visit was made to the pond where this infected *Ranunculus circinatus* was growing, and a good supply of roots was collected and fixed. At the same time roots of other associated water plants were collected.

On microscopic examination it was found that these roots were all heavily infected with a species of *Ligniera*. Since all the plants were growing in the same pond under conditions entirely suitable for infection from one another, it was thought extremely likely that all the roots were diseased with the same species of *Ligniera*.

About the same time roots of *Plantago major* growing in association with diseased roots of *Poa annua* were found to be infected with *Ligniera*.

From a consideration of the above facts together with the very close microscopic resemblance, doubt was cast upon the validity of the five species of *Ligniera* already described from the neighbourhood of Sevenoaks.

The following spring suitable plants were grown in the greenhouses at King's College, London, under conditions which rendered infection from *Ligniera* impossible, and after they had passed the seedling stage they were inoculated with roots heavily infected with suitable species of *Ligniera*.

At the same time, having a considerable quantity of diseased roots at my disposal, a microscopic examination was made in the hope of elucidating some cytological points which had not been very clearly described by previous workers.

Many roots showed stages in the formation of zoospores; these were recorded by Maire and Tison in *Ligniera radicalis* but were not observed by Schwartz in any of his material. The former workers, however, did not follow the subsequent fate of these zoospores.

Using living material, a study of the mode of infection by zoospores was made.

Inoculations.

In the early spring of 1925 seeds of the following plants were sown in sterile soil which had been autoclaved at a temperature of 130° C.: *Plantago major, Poa annua, Mentha Pulegium, Iris Pseudacorus, Veronica Beccabunga* and *Ranunculus circinatus.* The seedlings were potted forward, and by the end of May were strong healthy plants.

During the spring more examinations were carried out on the water plants growing in Knole Park, Sevenoaks, with the result that *Ligniera* was found to be present in *Ranunculus circinatus, Potamogeton natans, Iris Pseudacorus, Juncus articulatus, Ranunculus aquatilis* and *Callitriche stagnalis.*

From marshy ground in the same locality *Ligniera* was found in *Bellis perennis, Plantago major* and *Poa annua*; and in some ditches near by in *Alisma Plantago-aquatica.*

Near a large pond on Chislehurst Common, Kent, infected plants of *Mentha Pulegium* were also collected.

The infection of seedlings was carried out during May, 1925, and the experiments were divided into two groups, firstly the infection of healthy plants by diseased roots of the same host; and secondly by infecting plants with the disease by using the roots of a different host. Plants to be inoculated were knocked out of their pots and their roots broken and damaged; roots, also bruised and heavily infected with the disease were then placed in close association with them, and the whole repotted and kept well watered. In no case was the health of the host plant affected by this treatment, and in the great majority of cases the roots of the infecting plant also grew.

Towards the end of September all the plants were lifted and their roots examined for the presence of any fungus. In each series of experiments a certain number of plants, usually five, were kept as controls. The following table shows the number of plants which were infected and the results obtained.

The first column shows the number of plants inoculated and the second the number which became diseased.

From Table I it will be seen that *Ligniera* from the roots of seven different hosts was used for infection.

Out of thirty-two plants infected with the parasite from *Poa annua*, thirty-one developed the disease. No attempts were made to infect either *Mentha Pulegium* or *Veronica Beccabunga* owing to the small number of plants at my disposal. Diseased roots of *Plantago major* transmitted the disease to the roots of *Poa annua*, *Mentha Pulegium*, *Ranunculus circinatus*, *Iris Pseudacorus* and to healthy plants of the same species; out of twenty-eight plants twenty-six became infected.

Table I. *Plants inoculated.*

Material used in inoculation	*Plantago major*		*Poa annua*		*Mentha Pulegium*		*Ranunculus circinatus*		*Iris Pseudacorus*		*Veronica Beccabunga*	
*Poa annua**	10	10	5	5	—	—	12	11	5	5	—	—
*Plantago major**	3	3	5	4	5	5	5	4	10	10	—	—
Ranunculus aquatilis†	12	10	5	4	3	3	—	—	10	9	—	—
Mentha Pulegium‡	5	5	—	—	—	—	5	5	5	4	—	—
Callitriche stagnalis†	10	10	10	10	5	4	5	4	10	8	—	—
Veronica Beccabunga§	—	—	5	5	—	—	—	—	—	—	5	4
Juncus articulatus†	—	—	—	—	—	—	5	4	—	—	5	5
Controls (five plants)	0	0	0	0	0	0	0	0	0	0	0	0
Total plants	40	38	30	28	13	12	32	28	40	36	10	9

The plants were collected from the following localities:
* Marshy ground near Sevenoaks; † pond, Sevenoaks; ‡ Chislehurst; § Cambridge.

Heavily infected roots of *Ranunculus aquatilis* were used to inoculate thirty plants, and twenty-six of these became diseased. Only fifteen diseased plants of *Mentha Pulegium* were available for use as inoculants and five plants each of *Plantago major*, *Ranunculus hederaceus* and *Iris Pseudacorus* were used. As a result all except one plant developed the disease.

A large quantity of suitably infected material of *Callitriche stagnalis* was available and was used for experiments with *Plantago major*, *Poa annua*, *Mentha Pulegium*, *Ranunculus circinatus* and *Iris Pseudacorus*; out of forty plants thirty-six became infected.

Veronica Beccabunga, owing to its habitat, was not convenient to use for infecting other plants, and it was only tried with *Poa annua*; five plants were inoculated and all became diseased. Healthy plants of *Veronica Beccabunga* growing with diseased ones developed the fungus.

Good material of *Juncus articulatus* was difficult to obtain; five plants each of *Ranunculus circinatus* and *Veronica Beccabunga* were inoculated with diseased *Juncus* and all except one became infected.

In every case five control plants of each species were grown through the summer in sterile soil without being inoculated, and when examined in the autumn were all found to be free from *Ligniera*.

In all out of 165 plants used in the inoculation experiments, 151 had developed the disease after four months. That a few

should remain free from any infection is not surprising considering the methods used. As it was not possible to examine microscopically every root used as an inoculant, there was always the possibility that a few were free from any disease; this alone is sufficient to account for failure in the fourteen instances.

I was unable to carry out any satisfactory experiments with *Alisma Plantago-aquatica* owing to difficulty in keeping the plants alive in the greenhouse. Only one specimen was available for inoculation and it was grown in association with *Poa annua*. Its roots on examination later were found to be infected with *Ligniera*.

From the above results there is no doubt that, in the absence of any cytological distinctions, *Ligniera Junci*, *L. graminis*, *L. Bellidis*, *L. Menthae* and *L. Alismatis* are all the same species, which is capable, under suitable conditions, of infecting the roots of many Phanerogams growing in aquatic or marshy habitats.

During the summer of 1925 *Ligniera* was also found in the roots of *Galium verum* and *Ranunculus aquatilis* from Sevenoaks, in *Veronica Beccabunga* from Tintern, Mon., and in *Cerastium vulgatum* from Westerham, Surrey.

It is interesting to note that the fungus does not attack the roots of certain plants even when these are growing in close association with heavily infected roots of other species. A particular case which was brought to my notice was that of *Valeriana dioica* the roots of which were totally free from the disease, although plants of *Poa annua* and *Bellis perennis* growing close by were infected. The reason for this is not clear, but it may be connected with the relative acidity of the soil or to the presence of some substance in the roots themselves. Schwartz (20) pointed out that all the forms of *Ligniera* which he found, appeared in plants growing in marshy meadows, in which were springs breaking out at the junction of the Folkstone beds (Lower Greensand) with the Gault. In such places the soil is rich and heavily charged with iron salts. He also noticed that *Sorosphaera Veronicae* occurred much more frequently in such localities. *Valeriana dioica* is a chalk-loving plant and this difference in soil and situation may be an important factor in the infection of a host. In general, both *Ligniera* and *Sorosphaera* are found most commonly in rich alluvial soil containing an excess of iron. Experiments regarding the composition of the soil of infected plants are now in progress.

Microscopic Examination.

Methods.

A considerable quantity of suitably infected material was at my disposal as a result of the above inoculation experiments.

For fixation, Flemming's weaker solution, chrome acetic, Bouin's fluid, and Allen's modification of Bouin were used. Several corrosive sublimate formulae were also tried. These latter were not very satisfactory, tending rather to harden the roots. With alcoholic corrosive sublimate and picric acid considerable plasmolysis of the cells took place. Acetic-alcohol also proved too vigorous a fixative to be used. The most perfect fixation was obtained with Flemming's weaker solution and Bouin's fluid. No advantage was found in using Allen's modification, and eventually only Bouin's fluid was used. Large masses of fine roots were put in this fixative and after being air pumped were left for twenty-four hours. They were then washed in water and graded into 75 per cent. alcohol.

In selecting roots for examination very little notice was taken of the particular host plant used. Attention was chiefly paid to selecting those free from adhering detritus or any bacterial infection.

Two complete series of slides were made, one series stained in cotton blue and the other in Haidenhain's haematoxylin.

Small pieces of fixed root were examined microscopically and if heavily infected were put aside in 75 per cent. alcohol. In preparing cotton blue mounts the following method was found most satisfactory.

A solution of lacto-phenol, prepared by mixing one part each of lactic acid, glycerine, phenol and water was used as the mounting fluid. For staining a 0·3 per cent. solution of cotton blue was made up in lacto-phenol. Complete roots, a centimetre or so long, were removed from the alcohol and the spirit allowed to evaporate for a minute, a drop of lacto-phenol was then added and the slide was warmed until the fluid just began to steam. The lacto-phenol was drained off and replaced by a drop of the stain. The slide was warmed as before, and the cotton blue was allowed to act for twenty-five seconds. It was then drained off and the excess of stain washed off with lacto-phenol. When no more stain would come out the root was carefully rolled with a round pencil. In this way not only was the root flattened, but all excess of stain still in the vessels was squeezed out. The root was then placed on a clean slide further teased out with needles and mounted in a drop of lacto-phenol. Slides

were ringed with two coats of gum dammar in xylol, and then with one of black enamel.

In the case of the iron alum haematoxylin preparations the normal method was employed. The roots were left in 4 per cent. iron alum over night and stained in Haidenhain's haematoxylin for two hours. The excess of stain was removed with acid alcohol, and here again it was found that rolling the roots greatly helped the removal of excess of stain. The roots were carefully teased out before mounting in balsam.

Several attempts were made to section the roots. Material was brought up into wax rapidly in twenty-four hours, and also slowly; wax of different melting points was tried; but in every case the results were unsatisfactory, sections cut at all thicknesses from 2 μ to 10 μ showing the same broken appearance. I am informed by Dr E. J. Schwartz that he found the same difficulty in the preparation of sections.

The results obtained from squashed roots were so satisfactory, however, that it was found quite unnecessary to use sections even for the examination of nuclear divisions.

Life History.

The earliest stage which is usually recognisable in the roots of an infected plant is a small spindle-shaped uninucleated amoeba (Plate VIII, fig. 1). This consists of a granular mass of protoplasm with a large central nucleus having a large karyosome, which can be clearly seen in the photograph. The amoeba is naked.

Nuclear division takes place very soon, and is accompanied by increase in size of the amoeba which frequently sends out pseudopodia from the ends of the long axis (Plate VIII, fig. 2 and Plate IX, fig. 2).

This nuclear division has been called the "cruciform division" and is really a type of protomitosis, similar in many respects to that described in several of the Protozoa belonging to the Order Lobosa. It seems probable that the divisions are completed very quickly, as stages are very difficult to find. As a result of this previous workers have not given a very satisfactory account of the stages. It is not proposed in this paper to give a full account of what happens, as there are still a number of points which have to be cleared up before the complete account can be published. As far as the work has progressed, the process conforms almost exactly with that given by Nawaschin (14) in *Plasmodiophora*.

At the time of nuclear division the chromatin becomes formed into a plate with the karyosome, which may be occasionally double, situated at the centre (Plate IX, fig. 3). In polar view nuclei

at this stage are very difficult to distinguish from resting nuclei. There is next a gradual aggregation of the chromatin towards the periphery of the plate forming what, in side view, appears to be a dumb-bell. The plate then splits longitudinally and the two halves travel apart, the karyosome becoming drawn out in the centre. Gradually the karyosome also splits and travels with the chromatin to the poles, where the chromatin becomes curved round the outer periphery of the karyosome at each end of the spindle. During the whole process the nuclear membrane remains intact, though during the later stages it becomes more drawn out, and finally, after the reconstitution of the daughter nuclei, becomes invaginated at the centre and finally breaks in half forming the nuclear membranes of the daughter nuclei. There are, especially during the early stages, very clear signs of a spindle which is equal on both sides of the plate and not on one side only as was described by Nawaschin. Further work on this division is in progress.

Whether divisions are frequent is not known, but as very few are met with it is concluded that they are passed through very rapidly as the amoeba grows in size. This growth continues at the expense of the host tissue but normally there is no hypertrophy of the host cell. When, however, the amoeba is lying in a root hair a swelling is sometimes noticed, though infected root hairs which show no hypertrophy are more common.

In some cases, as was observed by Schwartz (22), two uninucleated amoebae fuse to form a single binucleated amoeba. There is no evidence to show that this fusion is in any way a conjugation, though it occurs only between very young amoebae.

The full grown amoeba may be compared with the plasmodium of the Mycetozoa, which it resembles very closely. The size of the full-grown amoeba is usually limited by the size of the host cell, though if this cell is very large the contents may become exhausted before the amoeba has completely filled it.

About this time further changes are observed in the nucleus. The karyosome which up to now has been the most characteristic feature of the nucleus, disappears and the amoeba is seen with chromatin only around the nuclear membrane. Osborn (16) working on *Spongospora subterranea* considered that the nuclei are reconstituted on fresh sites. As far as my observations go there seems no reason for making such a statement concerning *Ligniera*. No example has so far been observed in which the nucleus could be considered to become reformed on a new site. Plate IX, fig. 3, shows the typical condition met with at this stage. Chromatin which presumably passed out from the karyosome is to be seen lying in the cytoplasm. It will be noticed that the quantity of chromatin lying around the nuclear

membrane is about equal to that shown in a resting nucleus (Plate IX, fig. 2). From this fact it seems that in *Ligniera* only the karyosome passes into the cytoplasm during the akaryote stage. There is no reason for considering that during this stage there is any division of the nucleus. Whether all the chromatin sometimes passes into the cytoplasm could not be definitely observed. It is possible that its staining properties are changed at this stage, and were it possible to differentiate the chromatin part at any rate would be found inside the nuclear membrane.

After the akaryote stage has been passed through the amoeba is ready to form spores. The nuclei of the amoeba divide by two successive divisions which on account of the different size of the spindles are considered to be the reduction divisions. Each of the freshly formed daughter nuclei collects a small quantity of cytoplasm around it. Cell walls are then laid down around these areas and the whole plasmodium becomes converted into a mass of spores. Plate IX, fig. 4, shows an amoeba in the process of forming spores, the nuclei have divided and the cytoplasm is collecting around the daughter nuclei, some of which can be seen in the photograph. The spores themselves are shown in Plate IX, figs. 5 and 6.

Under certain conditions of moisture and temperature the amoeba, instead of forming ordinary spores, becomes cut up into much larger segments, each with a single unreduced nucleus. Around these masses vacuoles are formed and cell walls are laid down later. The nucleus then divides twice as in the case of spore formation. Plate VIII, fig. 4, shows in the two lower cells the large chromatin plate of the first division while in the upper segments are various stages in the second division. This division is a typical mitosis, and though it is not possible to count them, independent chromosomes come upon the equator of the spindle in metaphase. Each of these structures, which may be considered as a zoosporangium, thus contains four, presumably haploid nuclei. Around each of the nuclei a zoospore is organised, which at this stage appear tetrahedral in shape.

It has not been found possible to observe the further fate either of the ordinary spores or of the zoospores nor the method of escape from the host cells in which they are formed. Instances have been found in which some of these spores had escaped from a spore mass and it was noticed that the host cells near by were infected with very young amoebae; it suggests itself that the spores on germination penetrated the wall of the host cell and formed young amoebae in neighbouring cells. The same has been noticed in the case of the zoosporangia. A zoosporangium (Plate VIII, fig. 6) containing a single remaining zoospore was found, but the cells near by were not infected

with young amoebae. It was further noticed that these zoosporangia are found only in the epidermal cells and it seems most likely that the function of these motile spores is to spread the disease to the roots of other plants rather than of propagating it within the cells of the same root.

On one occasion when examining living material a large number of small zoospores were found swimming about in close association with roots containing empty zoosporangia. The size, shape and structure of these zoospores agreed exactly with the spores found in the zoosporangia, especially in the case where all but one of the zoospores had escaped, and the remaining spore had had room to assume its mature shape. (Cf. Plate VIII, figs. 1 and 6.)

These free-swimming zoospores were carefully observed. They are small spherical flagellated organisms about 4 μ in diameter with a single anterior flagellum about the same length as the diameter of the spore. There is a clear area around the anterior end and the nucleus is situated in the centre of the cell. Entrance of the zoospore was observed in one case. The particular root hair entered was swollen at the tip and the cell wall was already slightly broken. There was, however, no previous infection of the fungus either in the root hair or in the epidermal cell to which it was attached. Having entered the root hair through the broken tip the young flagellula swam actively for some considerable distance. For a period of a minute or so it would come to rest and then active motion would begin again. Finally, after reaching very nearly to the base of the root hair motion became spasmodic and finally only the flagellum continued to wave. The flagellum itself soon disappeared and the cell began to send out pseudopodia. The protoplasm became more granular and aggregated about the centre of the cell. The outline of the amoeba gradually became more even and after a short while the typical spindle-shape was assumed. Plate IX, fig. 1, shows two camera lucida drawings made of the living material. The active zoospore is shown outside the root hair and also very soon after entering it.

In the same piece of material several other root hairs were found containing one of these zoospores. These root hairs were not hypertrophied. No hole in the cell wall could be made out through which the zoospore had entered. These zoospores behaved in exactly the same way as described above. The whole process from the entrance of the zoospore to the formation of the spindle-shaped amoeba occupied about half an hour.

It is very probable that the original infection of roots is only by these zoospores and occurs solely through the root hairs, the ordinary spores being a method of tiding the fungus over bad conditions, and for propagation within the cells of the host plant.

Discussion.

From the evidence obtained from cross-inoculation of healthy plants of a number of Phanerogams with infected roots, it appears certain that the five species of *Ligniera* described by Schwartz are merely host varieties of the same species. It is proposed to call this *Ligniera Junci*, the name given by Schwartz to the first of the series he described.

Ligniera Junci is then the only species which is known to occur in this country, and is capable of infecting the roots of a number of aquatic and semi-aquatic flowering plants.

Ligniera pilorum, described from France by Fron and Gaillat (6), agrees with *L. Junci* both in its general life cycle, size of spores and zoospore formation. Its cytology has not been worked out, but, as it is found in the roots of *Poa annua* which is a plant frequently attacked by *L. Junci*, there seems little ground for considering it a new species. Fron and Gaillat lay stress upon the hypertrophy produced in the root hairs. If it is a feature of *L. pilorum* that it always causes hypertrophy, then it differs not only from *L. Junci* but also from all the described species of *Ligniera* and indeed cannot rightly be included in the genus which was formed to include only non-hypertrophying forms. If, as seems more likely, the hypertrophy of the root hairs is only occasional, and if the same fungus can be found in roots which have retained their normal size, then it agrees exactly with *L. Junci*, which sometimes attacks swollen root hairs. In such cases root hairs entirely free from the disease are frequently found, and there does not seem to be any direct evidence that this hypertrophy is due to the fungus.

Ligniera radicalis resembles *L. Junci* in its life cycle but differs from it in the size of the spores. While those of *L. radicalis* are 4–5 μ in diameter, those of *L. Junci* are 5–7 μ. On the other hand, *L. Junci* has been found in the roots of *Callitriche stagnalis*. I have not had the opportunity of examining any slides of *L. radicalis* so that I have no evidence for considering that *L. radicalis* and *L. Junci* are the same species, and in the absence of any definite information upon this point it is better to retain the name *L. Junci* for the British species. From the life history and cytology of the two forms, however, there is strong evidence that they are identical.

Ligniera verrucosa, described by Maire and Tison (11, 12) in the roots of *Veronica arvensis*, differs from *L. Junci* most markedly in the warted character of the walls of the spores. Further, instead of being massed together, the spores are more or less free in the host cells.

In their cytology all species of *Ligniera* are very similar.

Schwartz was never able to germinate the spores, nor did he find any stages of zoospore formation in his material. Maire and Tison, however, observed zoospore formation in the roots of *Ligniera radicalis*. Zoospores were also found by Fron and Gaillat. Careful measurements have shown that while the spores themselves are seldom more than 6 μ in diameter, the zoosporangia are often 9 or even 10 μ. Moreover, the wall of the spore is relatively thick while that of the zoosporangium is thin and frequently not spherical.

The life cycle cannot be completely known until the liberation and germination of the spores and zoospores has been followed. Prior to the formation of either spores or zoospores there are two nuclear divisions which appear to be heterotypic and homotypic, the equatorial plate of the first division being much larger than that of the second, as might be expected if this was a reduction division of a spore mother cell. Though these two divisions occur both before spore and zoospore formation, there is a difference in that whereas in zoospore formation the mitotic divisions occur after the segmentation of the amoeba into zoosporangia, in the formation of spores these divisions are completed before the amoeba becomes cut up into uninucleated portions. It is quite impossible to count the chromosomes during the divisions, and therefore there is no direct evidence that this is a reduction division, but by comparison with other types one is forced to such a conclusion. If this is the case the plasmodium at the time of spore formation must be diploid, and the spores and zoospores haploid. It is therefore necessary that at some point after spore formation there is a nuclear fusion. Schwartz suggested that there was a conjugation of the spores on germination. He did not, however, observe the actual process of germination of a spore, and there is still no direct evidence upon this point. In zoospore germination no fusion takes place as far as is known. Careful measurement of the single zoospore remaining in a zoosporangium (Plate VIII, fig. 6) shows that it is exactly the same size as the motile zoospore observed in the act of entering a root hair. This latter zoospore was watched till it assumed the shape of the uninucleated amoeba. It is therefore considered that no conjugation occurred between the liberation of the zoospore and the formation of the uninucleated amoeba within the cell of the new host. Conjugation of amoebae sometimes occurs, but it is common to find a single uninucleated amoeba lying alone in a host cell, and there is no evidence to show that this association of amoebae is in any way a fertilization. From the time the uninucleated amoeba begins to develop in the host cell until the commencement of the reproductive phase the nucleus divides by protomitosis, and

it is most improbable that any change in the number of chromosomes takes place here. The only other place where a nuclear fusion might occur is during the akaryote stage. No workers are at present clear as to what exactly happens during this nuclear change. Osborn considers that the nucleus may only lose the trophochromatin, while the rest of the chromatin still remains within the nucleus, but he realises that to suggest binuclearity in the Plasmodiophorales is unjustifiable without further evidence. It has been suggested that while a certain part of the chromatin passes into the cytoplasm the rest remains within the nuclear membrane, but owing to a difference in the chemical composition its response to stains is altered. Osborn held that there was at any rate an association of the nuclei at this stage and thought that very likely fusion did take place. Schwartz finds no ground for a nuclear fusion at this point in *Sorosphaera Veronicae,* and Maire and Tison, although they offer no suggestion as to where fusion does occur, are certain that it is not during the akaryote stage in *Ligniera.* Moreover, such a conclusion makes any comparison with the Mycetozoa increasingly difficult.

As has already been pointed out, the genus *Ligniera* stands apart from the rest of the Plasmodiophorales in not causing any hypertrophy of the host tissues. In all the other genera such hypertrophy occurs. According to Osborn, *Spongospora subterranea* does not produce any noticeable effect on the potato tuber which was the only organ on which he records the disease. Horne (7) on the other hand states that hypertrophy is produced as a result of the disease. This is further supported by Pethybridge (19), who figures cankerous growths on the roots of potato due to *Spongospora.*

The cytology of *Ligniera* conforms very closely with what is generally found in the Plasmodiophorales. Unfortunately spore germination has not been critically followed in any member of the order. Schwartz tried to germinate the spores of *Plasmodiophora Brassicae* without success. Duggar (3) gives an account of the germination of the spores of *Plasmodiophora* though he does not say whether they are his own observations. This description agrees very closely with what I have observed in *Ligniera Junci.* No one so far has germinated the spores of *Sorosphaera.* Massee (13) states that he had successfully germinated these of *Spongospora* and that the spore gives rise to a single myxamoeba, which is in agreement with my observations on the germination of zoospores in *Ligniera Junci,* and also with what is generally found in the Mycetozoa.

In *Plasmodiophora Brassicae* infection is through the growing point of the root, while both Schwartz (1) and Maire and Tison (9)

consider that in *Sorosphaera Veronicae* infection occurs through the growing apex of the stem. These workers and also Nawaschin find that the amoebae of *Sorosphaera* and *Plasmodiophora* have no power of penetrating cell walls. They think that propagation of the fungus throughout the host tissues is simply by division of the host cells. Such cannot be the case in *Ligniera Junci* where infected cells do not appear in any way to be associated together. Moreover, uninucleated amoebae are frequently found in epidermal cells where no empty spore cases are to be seen along the whole length of the root.

In 1912 Winge (23) described *Sorodiscus Callitrichis* in the stem of *Callitriche autumnalis*. The life history very closely agrees with that of *Sorosphaera Veronicae*. He examined the vegetative nuclei and found that in division the long axis of the chromatin plate was nearly always parallel. This is not the case in *Ligniera Junci*. Maire and Tison (9) do not consider that the vegetative soma is a plasmodium, since several times they have observed an amoeba in the metaphase and another in the anaphase of the sporogenous division in the same cell. I have always found that during the vegetative divisions all the nuclei divide simultaneously, which supports Nawaschin's view that a plasmodium is formed. This also agrees with Pavillard (17) who states that simultaneous division is constant throughout the endosporous Mycetozoa. Léger (8) has described a genus *Sporomyxa* which he related to the Plasmodiophorales living within the coelomic cavity of the imago of *Scaurus tristis*. From his figures it would seem that the nuclei of the amoeba all divide together.

The feature upon which classification is chiefly based is the shape of the spore masses. In *Plasmodiophora* the spores are free, in *Sorosphaera* they are in hollow spheres, while in *Sorodiscus* they are in flattened spheres and ellipsoids. In *Tetramyxa* they are found in tetrads, and in *Spongospora* in spongy masses. In *Molliardia* no spores have so far been found and, as Schwartz points out, when these are found it may be transferred to another genus. In *Sporomyxa* the spores are not arranged in any typical system but the individual spores are ellipsoidal.

It is desirable here to mention two further genera which have been described by Ferdinandsen and Winge, *Clathrosorus* (5) and *Ostenfeldiella* (4). *Clathrosorus* was described from some hypertrophied roots of *Campanula rapunculoides*. The material was only roughly fixed and was shrunken by the solution used. The authors claim to have seen both protomitosis and meiosis in their material; however, they give only a brief account of the life history. In *Ostenfeldiella* which was found causing hypertrophy of the stem of *Diplanthera Wrightii* these same authors

were even less fortunate in their material, as it was only preserved in alcohol. Apparently no cytological structure could be made out, and the figures show little more than masses of spores. Both genera, so far as the evidence goes, appear to be closely allied to *Spongospora*. It is much to be regretted that no further description of these genera is forthcoming.

In 1910 Němec (15) figured and described a fungus which he called *Sorolpidium Betae* and referred it to the Chytridiales. It was found in the roots of *Beta maritima*. As a result of this paper various attempts have been made to relate the Plasmodiophorales and the Chytridiales. From an examination of Němec's figures it would appear very likely that he was dealing with two independent fungi, the one a *Ligniera*, while the other resembles *Asterocystis* in the structure of the sporangium. Němec does not describe the cytology of his fungus very closely, and does not figure protomitosis. Chytridiaceous fungi are frequently met with in the roots of water plants infected with *Ligniera Junci*. During my examination of roots three species closely related to *Synchytrium* have been found; it is hoped to publish an account of these later. All these Chytridiaceous fungi differ from *Ligniera* in the much coarser and granular structure of the cytoplasm.

Cavers (2) was of the opinion that there was no direct relationship between the Chytridiaceae and the Plasmodiophoraceae, but gave them a common ancestry in the *Proteomyxa*.

The members of the Plasmodiophoraceae agree very closely in all important features. Moreover, the characteristic type of protomitosis is not found in any other group of fungi. It is, however, described in some of the Amoebae. Therefore it seems very desirable to keep the Plasmodiophoraceae quite separate from the Chytridiales, and other primitive fungi. If there is any relationship it is most likely through the Mycetozoa.

Systematic.

Since so much confusion has arisen concerning the systematic position of the genus *Ligniera*, and the species which compose it, it is desirable once again to give the generic and specific diagnoses put forward by Maire and Tison (12), omitting those which can no longer be considered as independent species.

Genus ***Ligniera*** Maire & Tison.

In cellulis hospitis immutatis parasitans; nec tumores gignens; schizogonia reducta; sporae in acervulos variiforme conjunctae.

L. verrucosa Maire & Tison.

Sporis crasse verrucosis, 4–5 μ diam., in acervulos plenos conjunctis. Habit. in *Veronica arvensi*.

L. radicalis Maire & Tison.

Sporis levibus 4–5 μ diam., in acervulos rarius cavos conjunctis. Habit. in *Callitriche stagnali*.

L. Junci (Schw.) Maire & Tison (= *Sorosphaera Junci* Schw., *S. graminis* Schw., *Ligniera graminis* Schw., *L. Bellidis* Schw., *L. Menthae* Schw., *L. Alismatis* Schw., *L. pilorum* Fron & Gaillat).

Sporis levibus 5–7 μ diam., in acervulos saepe cavos conjunctis. Habit. in radicibus plantarum aquatilium et palustrium.

Summary.

1. Cross-inoculation have been successfully carried out between the five species of *Ligniera* described by Schwartz.

2. The result of these experiments proves conclusively that *Ligniera Junci*, *L. graminis*, *L. Bellidis*, *L. Menthae* and *L. Alismatis* are only host varieties of the same species, for which it is proposed to retain the name *Ligniera Junci* on grounds of priority.

3. The life history of *Ligniera Junci* has been followed, and agrees in all essential points with that of the other two species *L. radicalis* and *L. verrucosa* described by Maire and Tison.

4. Reasons are given for considering that *Ligniera pilorum* is identical with *L. Junci*.

5. Zoospore formation has been observed in *L. Junci*. The process of infection by the zoospores in the root hairs has been followed and their movements have been followed from the time of entrance in the tip of the root hair to the formation of a spindle-shaped amoeba.

6. Reasons are given for considering that zoospores are the only method provided for the infection of fresh host plants; the spores being a resting stage for tiding the fungus over unsuitable weather conditions and for propagating it within the cells of the infected plant.

7. Reduction division has been observed at the time of formation of both spores and zoospores. Conjugation has never been found.

8. The stages in protomitosis have been followed, and the process so far as is yet known agrees with that described by Nawaschin in Plasmodiophora. Further work on this subject is in progress.

9. The genus *Ligniera* is a member of the Plasmodiophoraceae,

and has many points of relationship with the Mycetozoa. It differs from any other member of the group in not causing any hypertrophy of the host tissues.

10. The Latin diagnoses of the genus, and the recognised species as given by Maire and Tison are appended.

The work was done in the Botanical Department of King's College, London. I wish to take this opportunity of thanking Professor Gates for much helpful advice and criticism. I also desire to thank Dr E. J. Schwartz for his valuable assistance and advice throughout the investigation.

University of London,
King's College.

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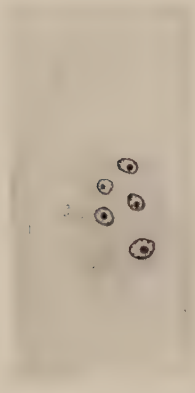

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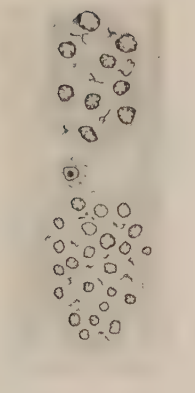

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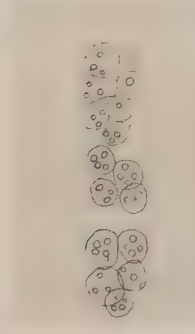

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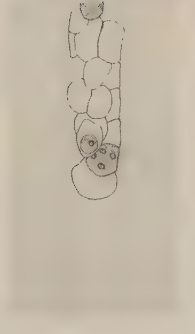

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LIGNIERA JUNCI

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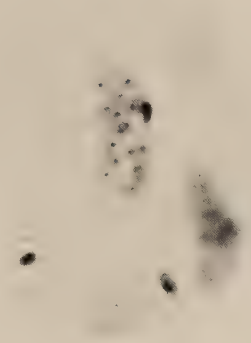

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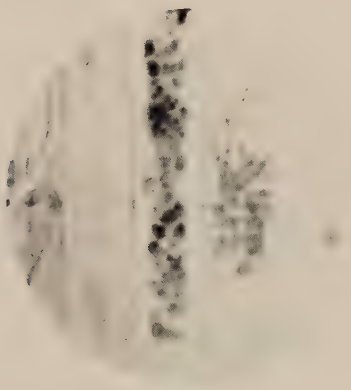

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LIGNIERA JUNCI

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DESCRIPTION OF PLATES.

The drawings were made with a camera lucida at table level, with a tube length of 160 mm. In the drawings a Koritska 2 mm. apochromatic objective (n.a. 1·30) was used with Zeiss compensating ocular 10 (figs. 1–3 and 5), with ocular 20 (fig. 4) and with ocular 7 (fig. 6). The photomicrographs were made with a Zeiss Seidentopf Photographic Eyepiece at tube length 115 mm. using a Zeiss 4·2 mm. (n.a. 0·65) (figs. 1, 2, 4 and 5), Zeiss 2 mm. (n.a. 1·4) (fig. 3) and Koritska 2 mm. (n.a. 1·3), (fig. 6).

PLATE VIII.

Fig. 1. Two stages in the entrance of the zoospore in the root hair. Both drawings were made on living material at short intervals from one another. × 650.
Fig. 2. A resting amoeba with five nuclei. Shows the chromatin around the nuclear membrane and the large conspicuous karyosome. × 650.
Fig. 3. The akaryote stage, showing the chromatin extruded into the cytoplasm, no karyosome, but the chromatin still remaining around the nuclear membrane. × 650.
Fig. 4. Two stages in the reduction division prior to the formation of zoospores. The plasmodium has already broken up into segments and then the nuclei have divided; the two on the left show the metaphase of the first division while those on the right are stages in the second division. × 1350.
Fig. 5. Zoosporangia showing the four resting nuclei of the zoospores. × 650.
Fig. 6 Empty zoosporangia, showing one with four zoospores still remaining and another with a single zoospore. Compare this zoospore with Fig. 1. × 480.

PLATE IX.

Fig. 1. A young uninucleated amoeba lying in a root cell, showing the large conspicuous karyosome. × 350.
Fig. 2. A resting plasmodium. × 350.
Fig. 3. Protomitosis in a plasmodium showing the typical "cruciform division." Owing to the difference of level a photograph of this stage can only show one or two nuclei in focus at one time. × 1750.
Fig. 4. Segmentation of the plasmodium into spores. The reduction divisions have taken place and the cytoplasm is being reconstituted around the nuclei. × 350.
Fig. 5. A mass of spores. × 350.
Fig. 6. Another mass of spores showing the structure of the wall. × 800.

NOTES ON A PYCNIDIAL FUNGUS ASSOCIATED WITH A DYING-BACK OF APPLE BRANCHES.

(With 7 Text-figs.)

By E. A. Southee and F. T. Brooks.

Field Observations.

A few years ago some Lord Derby apple trees at Meldreth, Cambridgeshire, were brought to our notice, large and small branches of which were gradually dying back. In the early stages of disease the bark was affected in long strips on one side

of the branch, with sometimes a sharp line of demarcation between the healthy and diseased tissues so that something in the nature of a canker was produced (cf. Fig. 1).

Occasionally, death of the bark in a long branch appeared to begin at the place of attachment of a spur which had been killed, but, more frequently, it could not be ascertained where

Fig. 1. Photograph of diseased bark on a Lord Derby apple tree. Natural size.

the disease had commenced. The disease frequently spread round the whole of the bark, and branches so affected died back. A characteristic feature of the dead bark was the manner in which the superficial tissues peeled away from the underlying parts.

A few trees of other varieties of apples such as Lane's Prince Albert and Cox's Orange Pippin have been seen to be affected by the same disease in other parts of the country, but the disease is uncommon.

The Fungus Associated with the Disease.

On dead parts of branches from which the superficial tissues had peeled away fructifications of a fungus were invariably found. These were small, more or less spherical bodies, greyish

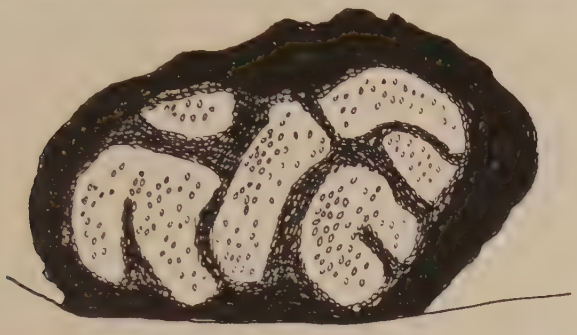

Fig. 2. Long. section through a naturally occurring stroma. × 100.

Fig. 3. Trans. section through a naturally occurring stroma. × 100.

black in colour, and about 1 mm. in diameter. These fructifications are stromata in which are embedded one or several pycnidia, more than one chamber being occasionally connected with a single ostiole (cf. Figs. 2 and 3).

The spores are formed from the extremities of short, tapering conidiophores, which line the base of the pycnidium. The spores

are oval or sub-globose in shape with rounded ends, have a large, conspicuous guttule, and are mostly hyaline, although a few (less than 1 per cent.) become light brown with age (cf. Fig. 4).

The spores vary in size from 9–14 × 7–10μ, averaging 10 × 7·5μ. The spores exude from the ostioles as creamy masses.

Although the fungus is confined to the bark at first, it ultimately penetrates the wood, causing it to become discoloured.

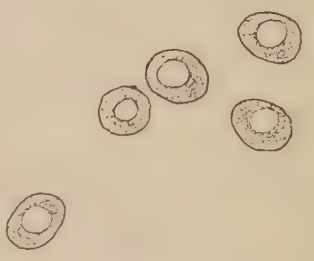

Fig. 4. Spores from a naturally occurring stroma. × 800.

Pure Cultures of the Fungus.

Cultures of the fungus were established from single spores upon potato agar, apple wood-extract agar, and apple twigs. The fungus grew well and produced fructifications on all these media. On sterilised apple twigs, however, the stromata bearing the pycnidia were often considerably elongated and projected from the surface as horn-like processes. Upon examining the pycnidia formed in culture it was seen that although some contained only the type of spore found in nature, others contained also large numbers of hyaline, straight, rod-like spores, 3–4 × 1 μ in size. These rod-like spores were never encountered in naturally-occurring pycnidia. As the cultures were continued, some pycnidia produced only the rod-like type of spore. The large pycnospores formed in culture frequently contained several guttules instead of the one characteristic of those found in nature.

Cultures of the fungus obtained from the junction of diseased and healthy tissues behaved in the same manner as those arising from single spores.

Germination of the Spores.

Spore germination was studied in hanging drop cultures of apple wood-extract and in thin films of apple wood-extract

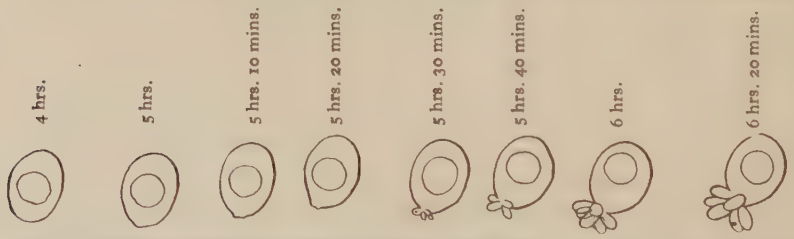

Fig. 5. Stages in the usual method of spore germination, the intervals after the time of placing the spores in the culture medium being indicated. × 1400.

agar spread on glass slides. The mode of germination was of two principal kinds, the commonest being of a peculiar nature.

After a few hours in the culture medium the spore swells and begins to show a slight prominence at one end. Shortly afterwards, bud-like protuberances arise in association with this prominence; these ultimately form secondary spores which break away from the mother spore. They are hyaline and measure 4·5–8·5 × 2–5·5 μ (cf. Fig. 5).

After another twenty hours these "budded," secondary spores

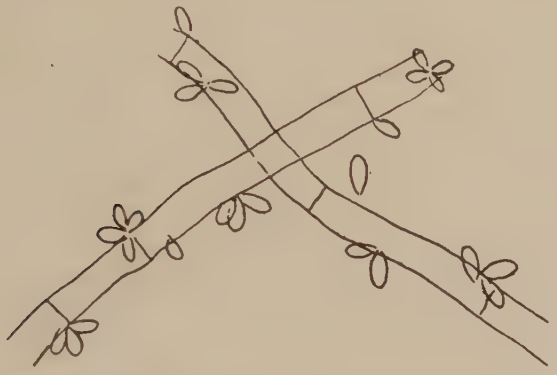

Fig. 6. Spores borne directly on the mycelium formed after germination. × 1400.

germinate from one or both ends to form hyphae. Meanwhile from the opposite end of the mother spore a normal germ tube usually arises, which develops into a mycelium. The mycelium, whether derived direct from a pycnospore or a "budded" spore, in turn forms other spores laterally, arranged singly or in groups, similar in size to the "budded" spores (cf. Fig. 6).

In the other type of germination, which is exceptional, two or three germ tubes of the usual kind arise from the pycnospores (cf. Fig. 7).

Fig. 7. Another, but exceptional, type of spore germination (after 24 hours). × 1400.

There are modifications in detail between these two types of germination.

We did not succeed in germinating the small, rod-like spores

Systematic Position of the Fungus.

There is considerable uncertainty about the systematic position of this pycnidial fungus. It probably belongs to the Sphaerioidaceae-Hyalosporae section of the Sphaeropsidales, although a few of the spores are brownish in colour. The choice of genus would appear to lie between *Dothiopsis*, *Cytosporella*, and *Dothiorella*. The naturally-occurring fructifications contain either one or several pycnidia, so that in this respect the distinction between *Dothiopsis* and *Cytosporella* breaks down. The distinction between *Cytosporella* and *Dothiorella* is based upon the size and shape of the spores, a not very satisfactory criterion.

As far as we are aware the small rod-shaped spores produced in some pycnidia under cultural conditions have not been described as occurring in either of the genera mentioned above, but as they do not occur under natural conditions, little stress need be laid on them. These small spores are similar in many respects to the "B" spores of many species of *Phomopsis*.

We are inclined to refer the fungus to the genus *Cytosporella*, especially as Mr J. Ramsbottom has kindly called our attention to a species *Cytosporella fructorum*, occurring on fruits of *Pyrus* in Belgium, which has been described by Él. and Ém. Marchal in the *Bulletin de la Société Royale de Botanique de Belgique*, LIV (1921), 125, and which agrees closely with the fungus now under consideration. Our fungus diverges slightly from that described by É. and É. Marchal in that the spores are not exactly globose and are slightly larger. It seems best, however, provisionally to assign the fungus to this species. An effort was made to obtain material of *Cytosporella fructorum* from Monsieur Marchal, but unfortunately it was not available.

Inoculation Experiments.

A large number of inoculation experiments with pure cultures of the fungus were carried out in wounds made in branches of Lord Derby apple trees, but in no case did infection result. It was invariably found that the fungus, after penetrating the tissues slightly, soon ceased to develop further, and the wound was ultimately occluded. The pathogenicity of this fungus is therefore still in doubt. Observations in the field indicated that the fungus was behaving as a slow-growing parasite, and it may be that it can only act parasitically in trees which have been weakened by some other cause. Recent work in the pathology of fruit trees indicates that there are several fungi which act destructively only when the trees are in a debilitated condition. The Lord Derby apple trees on which the fungus

chiefly occurred were growing amongst rank grass in a rather poorly drained, heavy soil overlying chalk. Such conditions may have led to considerable weakening of the trees, allowing the fungus to attack them.

Summary.

1. A die-back of the branches of Lord Derby apple trees in Cambridgeshire is described.

2. A pycnidial fungus is constantly associated with this disease. There is some uncertainty about the identity of the fungus, but it is provisionally named *Cytosporella fructorum*, a species which Él. and Ém. Marchal have described as occurring on fruits of *Pyrus* in Belgium.

3. The methods of germination of the pycnospores are described. The commonest mode of germination is peculiar in that secondary spores are budded off from one end of the pycnospore.

4. Under cultural conditions many pycnidia give rise to small rod-like spores which have not been found in nature.

5. Inoculation experiments with the fungus were unsuccessful in reproducing the disease. It is likely that the fungus behaves as a parasite only when the trees are already weakened from some other cause.

THE INHERITANCE OF SPORE SIZE IN COPRINUS STERQUILINUS.

(With 2 Text-figs.)

By W. F. Hanna, M.Sc. (Alberta).

I. Introduction.

The possibilities of increasing the food value and productivity of the higher plants by selection of superior individuals must have been recognised in a general way by all primitive agricultural peoples. However, the fundamental principles which are involved in the process of selection have only recently been studied. Johannsen's* classical researches on pure lines form one of the foundation stones upon which the present-day conception of selection has been built. He chose for his experiments a certain variety of the common garden bean, *Phaseolus vulgaris nana*, known as the Princess bean. In 1901, he planted a number of bean seeds of different sizes and known weights. At the end of the season each plant was harvested separately and an exact record was kept of the weight of each seed. When the weights

* Johannsen, W. Elemente der exakten Erblichkeitslehre. Jena, 1909.

of the mother beans were compared with the weights of the progeny, it was evident that the original population from which the mother beans had been selected was a mixed one and that selection had resulted in the sorting out of a number of already existing types or "pure lines." Johannsen then proceeded to determine the effect of the selection of plus and minus variants within his pure lines. In these experiments he employed nineteen pure lines, and the work was carried on for six years, from 1902 to 1907 inclusive. Each year, every pure line was represented by two lots of plants, one a plus strain grown from large beans and the other a minus strain grown from small beans. Continued selection, however, failed to produce permanent departure in either direction and the offspring of the plus and minus variants exhibited complete regression to the mean of the particular line. A series of similar experiments using the character length and breadth of seed gave the same result. Johannsen, therefore, concluded that individual variations within a pure line are not inherited and that selection within such a line is without effect.

Johannsen's experiments and those of other investigators have shown that in the higher plants the species must be regarded as consisting of various groups of individuals which possess a general similarity but which, nevertheless, differ from one another in respect to minor heritable characters. Environmental conditions may bring about further variation in the individuals making up each group or pure line; but, on the basis of Johannsen's work, modifications of this kind will not be transmitted to succeeding generations and will no longer appear when the influences which brought them into existence have ceased to operate. Heritable modifications within pure lines, therefore, can arise only by mutation.

Biologic forms are known to be of relatively common ocurrence in many species of parasitic fungi. Furthermore, Arthur*, Gäumann†, Levine‡, and others have shown that biologic specialisation is accompanied in many instances by a certain degree of morphological differentiation. By using single-spore cultures Brierley§ demonstrated the existence of a number of races, morphologically distinct, within species of *Botrytis*, *Penicillium*, and *Stysanus*. On the basis of such evidence, we may conclude that species of fungi are not homogeneous in com-

* Arthur, J. C. Cultures of Uredineae in 1916 and 1917. Mycologia, IX (1917), 295–312.

† Gäumann, E. Ueber die Formen der *Peronospora parasitica* (Pers.) Fries. Beih. z. Bot. Centr. (Abth. 1), XXXV (1917–18), 395–533.

‡ Levine, M. N. A statistical study of the comparative morphology of biologic forms of *Puccinia graminis*. Jour. Agr. Res. XXIV (1923), 539–567.

§ Brierley, W. B. Experimental studies in the specific value of morphological characters in the fungi. Proc. Lin. Soc. London, Oct. 1918.

position but, like those of the higher plants, are made up of a number of separate races or pure lines.

Very little experimental evidence is available as to the value of continued selection within pure lines of fungi. On *a priori* grounds it might be expected that selection in fungi would obey the same general laws as in the higher plants, *i.e.* that continued selection within a pure line would fail to bring about any permanent departure in either morphological or physiological characters. It is well known that fungi possess a high degree of variability and that, under cultural conditions, many species are extremely unstable. The possibilities of selection in fungi, therefore, would seem to be worthy of serious consideration. Burkholder* has recently shown that long-continued culturing of *Fusarium Martii Phaseoli* on artificial media may bring about changes in both morphological and physiological characters, which persist for some time after the fungus has been again transferred to its host plant. The strain with which he worked was isolated from a diseased bean root, and the first culture was made from a single spore. Six years of culture on artificial media was effective in bringing about distinct changes in the colour of the mycelium and in the size and number of the conidia produced and, in addition, the fungus had lost much of its former virulence. When this attenuated form was allowed to infect the bean plant and was then re-isolated, its original characters were partly restored and, after two such passages, the organism was found to possess all its former virulence. Rosenbaum†, on the contrary, found that selection of large conidia of *Phytophthora infestans* for five generations was ineffective in producing any change either in the size of the conidia or in the relative numbers of large and small conidia.

La Rue has carried out extensive experiments on the effect of selection in *Pestalozzia Guepini*‡ and *Helminthosporium teres*§. All the cultures used by him originated from single spores. Selections according to progeny in *Pestalozzia Guepini* were made for length of spore for ten generations and for length of spore appendages for twenty-five generations; but, at the end of the selection period, no permanent modification had been

* Burkholder, W. H. Variation in a member of the genus *Fusarium* grown in culture for a period of five years. Amer. Jour. Bot. XII (1925), 245–253.

† Rosenbaum, J. Studies of the genus *Phytophthora*. Jour. Agr. Res. VIII (1917), 233–276.

‡ La Rue, C. D. The results of selection within pure lines of *Pestalozzia Guepini* Desm. Genetics, VII (1922), 142–201.

§ La Rue, C. D. The results of selection within pure lines of the genus *Helminthosporium*. Paper read before Amer. Assoc. for the Advancement of Science, Dec. 1925.

brought about in respect to either of these characters. Similarly, when individual large and small spores were selected, in one experiment for six generations and in another experiment for ten generations, no increase or decrease in the average size of the spores was effected. The experiments with *Helminthosporium teres* were carried out for thirty generations, and the selections were made on the basis of spore length. A long line was propagated from the longest individual spore of each generation, and a short line was propagated from the shortest individual spore of each generation, while a third unselected control line was propagated from spores taken at random. The mean spore length of each generation was obtained by measuring 150 spores from each culture. Throughout the experiment the average length of the spores in the three lines paralleled one another in a remarkable manner, and the selecting of long and short spores seemed to be without effect in altering the average spore length of the species.

From the experimental evidence that is available it would appear that the spore characters of fungi are comparatively stable and cannot be readily altered by selection. Final conclusions, however, will be warranted only after a careful study of many groups of fungi.

The size, shape, and colour of the reproductive bodies of the higher fungi are generally regarded as reliable characters for systematic purposes. In the classification of the Agaricaceae much importance is placed upon macroscopic characters, such as the shape and colour of the pileus and the attachment of the gills; but spore size is also known to be of great value in the identification of species, and in most modern works on mycology spore measurements are included in the description of each species. It is well known that there may be considerable variation in the size of the spores collected from a single fruit body. Differences have also been noted in the average size of spores from different fruit bodies of the same species. Buller* determined the average diameters of the spores of three wild fruit bodies of *Amanitopsis vaginata* by measuring 50 spores from each fruit body and found them to be 10·19 μ, 10·87 μ, and 11·65 μ respectively. When systematic works are consulted for the spore dimensions of a particular species, it is frequently found that there is considerable disagreement between the measurements recorded by different authors. Differences of this kind might reasonably be expected if the spore sizes given were based on the measurement of a few spores from a single fruit body. There is also the possibility that within species of the Agaricaceae there may exist numerous strains or pure lines

* Buller, A. H. R. Researches on Fungi, 1 (1909), 161.

each having a characteristic size of spores. As far as the writer is aware, however, no experiments on the inheritance of spore size in this group of fungi have been made. The present paper is a contribution to this subject.

The fungus selected for experiment was *Coprinus sterquilinus*, a large coprophilous species which occurs commonly on horse dung in both Europe and North America. It has been described and fully illustrated by Buller*. Miss Mounce† has shown that this species is homothallic, a fact since confirmed by Brunswik‡.

In the experiments to be recorded later, *Coprinus sterquilinus* was successfully cultivated in monosporous culture for several successive generations. In one series of experiments each generation was started from as small a spore as possible, and in another series of experiments each generation was started from as large a spore as possible. Both series of experiments were carried out with a view to determine whether or not the ordinary fluctuating variations in spore size are inherited.

II. Methods.

In studying the effect of selection on a particular organism, careful consideration must be given to the genetic purity of the material from which selections are to be made. If a normally self-fertilised species such as the bean is employed, the progeny of a single seed may be regarded as a pure line. If, however, a cross-fertilised species is employed, selection of self-fertilised individuals for several generations will be necessary before an approximately homozygous condition is reached.

In view of recent progress in our knowledge of sex in the higher fungi, *Coprinus sterquilinus* may be regarded as a particularly suitable species for use in an experimental study of the inheritance of spore size. The work of Miss Mounce§, supplemented by the nuclear studies of Brunswik‖ has shown that a single spore of *Coprinus sterquilinus*, when germinated on dung agar, gives rise to a haploid mycelium characterised by simple septa and isolated nuclei and that, after a few days, this haploid mycelium spontaneously becomes diploid, the diplophase being indicated by the presence of clamp connections

* Buller, A. H. R. Researches on Fungi, III (1924), 177–257.

† Mounce, Irene. Homothallism and the production of fruit bodies by monosporous mycelia in the genus *Coprinus*. Trans. Brit. Mycolog. Soc. VII (1921), 198–217.

‡ Brunswik, H. Untersuchungen über die Geschlechts- und Kernverhältnisse bei der Hymenomycetengattung *Coprinus*. K. Goebel's Botanische Abhandlungen, V (1924), 1–152, Jena.

§ Mounce, Irene. Loc. cit. pp. 203–205.

‖ Brunswik, H. Loc. cit. pp. 15–19.

and the occurrence of the nuclei in pairs. The nuclei in this diploid mycelium divide conjugately. After a few weeks the diploid mycelium gives rise to perfect fruit bodies. In each young basidium there are two nuclei of opposite sex which fuse together. The fusion nucleus divides twice, as shown by Buller*, and the four nuclei pass upwards through the sterigmata into the four spores. Thus the life history of the fungus passes through all its possible stages beginning with a single spore; in other words, *C. sterquilinus* is homothallic. The progeny of a single spore of *C. sterquilinus*, therefore, may be regarded as a pure line.

Cultures of *C. sterquilinus* were made from spore deposits collected from a number of wild fruit bodies which appeared on dishes of horse dung kept in the laboratory. A spore deposit was obtained as follows. When a fruit body had begun to shed its spores, the pileus was removed by cutting through the stipe at the level of the pileus periphery. The pileus was then pinned through its centre to a small cork which had previously been attached with sealing wax to a circular glass plate. A sterilised glass slide was placed in the bottom of a crystallising dish and the glass plate, with the pileus suspended on its lower side, was placed as a cover over the dish. In this way the pileus came to hang at a height of from two to three inches immediately above the glass slide. As soon as the slide had become coated by a thin spore deposit—of such a nature that, when viewed under the microscope, the spores were seen to be fairly close together although not touching one another—it was removed, labelled, and placed in a cardboard case.

Single spores were removed from the dry spore deposit on a glass slide by the dry-needle method† and were sown singly in hanging drops of nutrient gelatine having the following composition:

Dextrose	10 gm.	Sodium chloride	5 gm
Peptone	10	Gelatine	100
Beef extract	5	Water	1000 cc.

Out of a total of 433 spores selected from 50 different fruit bodies and placed in hanging drops of gelatine for germination, 101 or 23·3 per cent. germinated. There was considerable variation in the viability of spores from different fruit bodies. In some fruit bodies the number of spores germinating was higher than 23·3 per cent., while in other fruit bodies the percentage was much lower than this and the spores could be induced to

* Buller, A. H. R. Researches on Fungi, III (1924), 208.

† Hanna, W. F. The dry-needle method of making monosporous cultures of Hymenomycetes and other fungi. Ann. Bot. XXXVIII (1924), 791–794.

germinate only with the greatest difficulty. In general, the small spores were less viable than the large ones. Of the 433 spores selected and sown 229 were between 10·7 μ and 16·7 μ in length, and of these only 16·2 per cent. germinated; the remaining 204 spores were between 17·2 μ and 23·3 μ in length, and of these 31·4 per cent. germinated. The larger spores, therefore, germinated about twice as well as the smaller ones.

Miss Mounce* has already referred to a paper by Miss Baden† on the germination of spores of *C. sterquilinus*. Miss Baden found that the spores of *C. sterquilinus* germinated only when certain bacteria about 1·2 μ in length and 0·8 μ in breadth were present in the culture medium. Other longer bacteria inhibited the growth of the mycelium. She states that "hanging-drop cultures were made with and without the bacteria. In those with the bacteria germination took place within twenty-four hours, but it never occurred at all without them. These experiments seem to show that the bacteria are in some way necessary for the germination of the spores." She further found that the spores of *C. sterquilinus* did not mature until about three weeks after they had been shed but that if dried for two days at 40° C., they would germinate at once. This she regarded as an adaptation on the part of the spores to retard germination until the substratum had become fairly dry and such fungi as *Mucor* had disappeared.

The results which Miss Baden obtained have never received any confirmation. In 1911, A. H. R. Buller and S. G. Churchward carried out a series of experiments‡ which proved conclusively that the spores of *C. sterquilinus* will germinate satisfactorily in a number of sterile synthetic media. Later, Miss Mounce § germinated the spores and obtained fruit bodies without difficulty under sterile conditions of culture. In the present study, many strains of *C. sterquilinus* have been grown in pure culture throughout several generations. The spores were found to germinate at room temperature in hanging drops of *sterile* nutrient gelatine or dung agar. Moreover, no difference was observed in the germination of spores which had just been shed and those which had been kept for several weeks. On one occasion, ten spores which had just been shed were placed in hanging drops of sterile nutrient gelatine and within twenty-four hours all had germinated. My own observations, therefore, confirm those of Buller and Churchward and of Miss Mounce; they seem

* Mounce, Irene. Loc. cit. p. 213.

† Baden, M. L. Observations on the germination of spores of *Coprinus sterquilinus* Fr. Ann. Bot. XXIX (1915), 135–142.

‡ Communicated to me in MS. by Professor Buller.

§ Mounce, Irene. Loc. cit. pp. 203–205.

to provide convincing evidence that Miss Baden was entirely mistaken in concluding (1) that the spores of *C. sterquilinus* germinate only in the presence of certain bacteria, and (2) that they are not ready to germinate when they are shed.

Fruit bodies were obtained by transferring the monosporous mycelia to dishes of sterile horse dung. Small crystallising dishes, five inches in diameter and three inches in depth, were found to be suitable for the purpose; they were half-filled with horse dung, covered with Petri-dish covers, and autoclaved for one hour at fifteen pounds pressure. A small piece of sterile dung agar was placed on the dung in each crystallising dish and a hanging drop of gelatine with its monosporous mycelium was transferred to the dung agar. By this method fruit bodies were generally obtained twenty-five days from the time of spore germination, although cultures were frequently observed to fruit in twenty-two days. No culture fruited in less than twenty-two days.

All spore measurements were made with the Poynting Plate Micrometer. This instrument has been described and illustrated by Buller* who was the first to employ it in biological research. The instrument used for the experiments recorded in the present paper was attached to a Watson microscope equipped with a mechanical stage which could be rotated when necessary. The mechanism for rotation was not present in the original instrument used by Buller. The Poynting Plate Micrometer combines speed of manipulation with a high degree of accuracy and has given most satisfactory results throughout the present work.

The method finally adopted for measuring spores was somewhat different from that which is generally employed and, therefore, will be described in detail. In preliminary experiments the spores were mounted in a drop of water on a glass slide, covered with a coverglass, and measured wet; this is the method usually adopted. It was noticed, however, that the true lengths of some spores were difficult to obtain owing to the fact that, in water, the long axes of the spores were not parallel to the face of the glass slide. If the cover glass was pressed down slightly with a view to remedying this defect, the spores often became appreciably compressed, thus bringing about a change in the ratio of length to breadth. It was then found that *the spores could be measured much more accurately when dry than when wet.* As is recorded elsewhere, the author working in conjunction with Buller† discovered that spores of *C. sterquilinus* which have fallen from a pileus and have settled upon a dry glass slide always have their long axes parallel to the surface

* Buller, A. H. R. Researches on Fungi, I (1909), 158–163.

† Ibid. III (1924), 224–230.

of the slide, and that each spore is held in this position by a thin colourless adhesive layer on its more rounded side which is always directed towards the surface of the glass. These dry spores never alter in shape or position. When viewed under the microscope, they appear perfectly regular in outline and are perfectly still, so that they can be measured with great accuracy. All spore dimensions which are recorded in the following experiments are based on the measurement of dry spores collected on glass slides.

The dimensions which are given for the dry spores may be compared with similar measurements presented by other workers for spores mounted in water by referring to the following table:

Table I.

Condition of spores	Mean length of spores in microns	Mean breadth of spores in microns
Dry	17·4	12·6
Wet	18·6	11·8

The sizes given in Table I were calculated from the measurements of 36 spores. Each spore was measured separately on the dry slide and was then transferred with the needle to a drop of water and measured again. On the basis of these measurements, a calculation shows that dry spores, on being wetted, increase in length 6·9 per cent., and decrease in breadth 6·4 per cent.; but that the product of their length and breadth remains practically constant.

In comparing the sizes of the spores from different fruit bodies, lengths only have been considered. The breadth of the spores, however, may be calculated from the data in Table I by taking the ratio of length to breadth of dry spores as 1 : 0·72 and that of wet spores as 1 : 0·63.

The number of measurements which must be made to determine accurately the average length of the spores from a given fruit body depends upon the variability in size of the spores which are to be measured. If the range of variability is small, a few measurements may suffice; while, if the variability is great, a correspondingly larger number of measurements will have to be made. For two fruit bodies, one with an average spore length of 18·7 μ, and the other of 18·4 μ, the probable error in the measurement of 100 spores was found to be $\pm$ 0·08 μ. When groups of 20 spores were measured from the same two fruit bodies, the experimental error in determining the average spore length was found to be as high as $\pm$ 0·5 μ. In the following experiments the average lengths recorded for spores of different fruit bodies are based on the measurement of 100 spores

from each fruit body. When this number of spores is employed, the error in determining the average length should be small even for fruit bodies with a wide range of variability in spore size.

III. Variation in the Size of Spores of Different Wild Fruit Bodies.

An examination of spore deposits of *Coprinus sterquilinus* collected from wild fruit bodies, which appeared upon horse dung cultures in the laboratory, showed that a wide variation in size may be found among the spores from a single fruit body. Considerable differences were also observed in the mean size of the spores produced by individual fruit bodies. The extent of these variations is brought out in Table II which gives the mean spore size and the limits in variation in spore size for five wild fruit bodies.

Table II.

Fruit body	Mean spore length in microns	Range of variation in spore length in microns
1	19·9	17·7–21·9
2	18·7	16·3–21·4
3	18·7	13·5–21·4
4	16·1	13·0–19·5
5	15·9	11·6–18·1

Table III.

Authority	Length in microns	Breadth in microns
Baden†	15–18*	8–12
Carleton Rea‡ ...	14–23	9–14
Kauffman§	18–25	—
Murrill‖	18	12
Ricken¶	18–22	12–14

* The length actually given is 0·15 mm.–0·18 mm., but this is obviously intended for 0·015 mm.–0·018 mm.

† Baden, M. L. Loc. cit. p. 140.

‡ Rea, Carleton. British Basidiomycetae, p. 501. Cambridge, 1922.

§ Kauffman, C. H. The Agaricaceae of Michigan, Lansing, I (1918), 211.

‖ Murrill, W. A. Mycologia, III (1911), 167.

¶ Ricken, A. Die Blätterpilze, I, 57. Leipzig, 1915.

As may be seen from an inspection of Table II, the limits of variation in the lengths of individual spores from different fruit bodies are from 11·6 μ to 21·9 μ, while the mean lengths of the spores from different fruit bodies range from 15·9 to 19·9 μ. Converted into lengths of wet spores with the help of the data for dry and wet spores given in connection with Table I, the lengths just referred to become 12·4 μ to 23·4 μ, and 17·0 μ to 21·3 μ respectively. It is, therefore, quite evident that *the measurement of any number of spores from a single fruit body*

would be entirely insufficient to give a true picture of either the range in variation or the mean size of the spores for the species as a whole.

Reference to a number of works showed that mycologists are by no means agreed as to the size of the spores of *C. sterquilinus*. The size of the spores, as given by several authorities, is set down in Table III.

The range of spore length given is from 14 μ (Carleton Rea) to 25 μ (Kauffman). Whether the limits of variation given by these authorities apply to the spores of a single fruit body or to those of several fruit bodies is uncertain and, in the absence of such information, these data are of limited value.

IV. Experiments on the Inheritance of Spore Size.

To determine whether or not individual variations in spore size are inherited in succeeding generations, single spore selections were made from the wild fruit bodies Nos. 1 to 5 of Table II and from another fruit body not included in this table. The results of these experiments are shown in Table IV. From fruit body No. 1, large spores were selected for five generations; from fruit body No. 2, large spores were selected in three lines of experiment and small spores in two lines for five generations; from fruit body No. 3, small spores were selected for three generations; from fruit bodies Nos. 4 and 5, small spores were selected for two generations; from fruit body No. 6, small spores were selected for five generations. The original spore deposit of fruit body No. 6 was unfortunately lost before measurements from it could be made.

As may be inferred from a study of Table IV, no particular relationship appears to exist between the size of the spore selected and the mean size of the spores from the resulting fruit body. Furthermore, although the original fruit bodies from which selections were made varied considerably in respect to the size of their spores, these differences do not appear to have been inherited as they have not been consistently retained in the succeeding generations.

The results presented in Table IV may be studied more easily by comparing the mean size of the spores from all the fruit bodies produced by monosporous mycelia arising from large spores with similar data for all the fruit bodies produced by monosporous mycelia arising from small spores. This information is summarised in Tables V and VI.

The mean length of the spores from the 21 fruit bodies resulting from large-spore selections was 18·1 μ, while an equal number of fruit bodies resulting from small-spore selections had a mean spore length of only 17·7 μ. At first sight these data seem to

Table IV. *The effect of selecting large and small spores of* Coprinus sterquilinus *throughout several successive generations.*

( ) indicates length of mother spore in microns. O indicates mean length of 100 spores from the progeny fruit body.

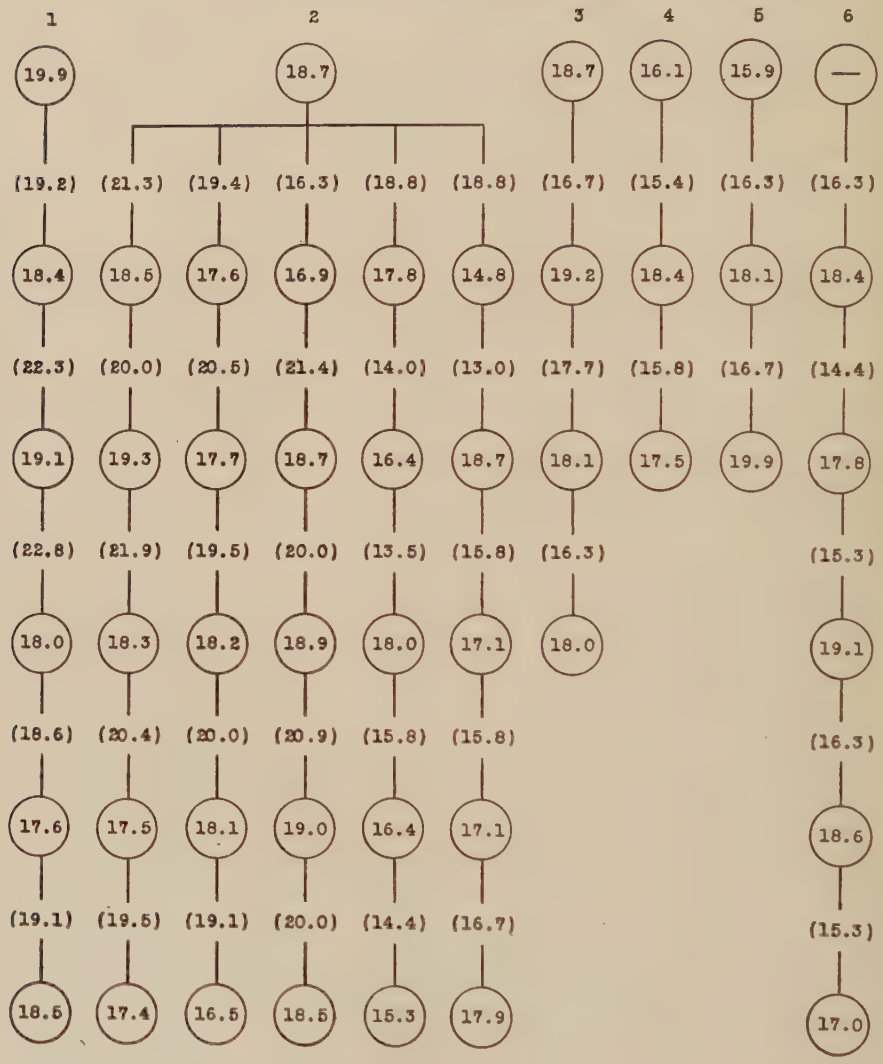

Table V. *Fruit bodies produced by monosporous mycelia arising from large spores.*

Generation	Number of fruit bodies	Mean spore length of progeny fruit bodies in microns
1	5	17·4
2	4	18·7
3	4	18·4
4	4	18·1
5	4	17·7

Table VI. *Fruit bodies produced by monosporous mycelia arising from small spores.*

Generation	Number of fruit bodies	Mean spore length of progeny fruit bodies in microns
1	5	18·2
2	6	18·1
3	4	18·1
4	3	17·4
5	3	16·7

suggest that the selection of small spores has had a slight effect in reducing the size of the spores in the progeny. However, when considered in relation to the differences in the mean size of the spores from individual fruit bodies, the effect of selection in modifying spore size appears to be without significance.

The coefficient of correlation between the size of the mother spores and the mean size of the spores produced by the progeny fruit bodies was calculated by Karl Pearson's formula. The value obtained was $0{\cdot}257 \pm 0{\cdot}097$. When considered in relation to the probable error, the evidence of correlation is very slight. The following conclusions may therefore be drawn: (1) the original wild fruit bodies from which selections were made did not represent a number of strains possessing spores of a particular size, and the differences in spore size which they exhibited were merely the result of fortuitous variations which were not inherited in succeeding generations; and (2) the continued selection of large and small spores for five generations did not result in any material increase or decrease in the size of the spores produced by the progeny fruit bodies.

In view of the experiments which have just been described, spore size may be regarded as a stable character for systematic purposes provided sufficient consideration is given to variability in the size of spores from individual fruit bodies.

The large number of spore measurements which were made in studying the inheritance of spore size in *C. sterquilinus* provide very suitable material for an analysis of the variations in spore size in this species, and an account of these results will now be given.

V. Variation in Spore Size during the Spore-discharge Period.

Considerable variation may be found in the mean size of the spores collected from a fruit body at different times during the spore-discharge period. Measurements of the mean length of spores collected from three fruit bodies at the beginning, middle, and end of the spore-discharge period are given in Table VII.

Table VII.

Period of spore discharge	Fruit body *A* Mean length of spores in microns	Fruit body *B* Mean length of spores in microns	Fruit body *C* Mean length of spores in microns
Beginning ...	17·4	17·3	18·7
Middle	18·4	17·7	18·7
End	20·0	16·9	19·1

From the data embodied in Table VII it is clear that there is considerable variation in the mean length of the spores produced by a fruit body at different times during the spore-discharge period. While in fruit bodies *A* and *C* there is an increase in the size of the spores produced at the end of the spore-discharge period, in fruit body *B* there is a decrease. To what these differences are due is by no means evident. That spores which develop under conditions of desiccation may be subnormal in size has been shown by Cotton* who found that there was a gradual diminution in the size of the spores shed by a pileus of *Stropharia semiglobata* which had been severed from its stipe and placed in the warm dry air of a room: the spores collected during the first hour of spore discharge measured 18 μ in length, while those collected in the eighty-third hour measured only 12 μ in length and were pale in colour. In the present investigation the spore deposits of *Coprinus sterquilinus* were collected from pilei suspended in closed crystallising dishes, so that very little drying of the gills, if any, could have taken place. Moreover, in *Coprinus sterquilinus*, as shown by Buller†, the spores on the gills all attain their maximum size before the pileus expands and begins to shed its spores from below upwards. It is clear, therefore, that transpiration during the spore-discharge period cannot have affected the size of the spores liberated. The spores in the Coprini ripen on each gill in succession from below upwards and are shed from below upwards; so that the spores collected from a fruit body at the beginning, middle, and end of the spore-discharge period are derived respectively from the

* Cotton, A. D. On the production of imperfectly developed spores in the Agaricaceae. Trans. Brit. Mycolog. Soc. IV (1914), 298–300.

† Buller, A. H. R. Researches on Fungi, III (1924), fig. 73, p. 184.

lower parts, middle parts, and upper parts of the gills. Possibly, therefore, the variations in spore size given in Table VII were due to an irregular flow of food materials to the hymenium during its development.

VI. A Correlation between the Size of the Spores and the Width of the Pileus.

The size of the spores produced by a fruit body is correlated to some extent with the size of the pileus of the fruit body. Buller* found that the spores of dwarf fruit bodies of *Coprinus lagopus*, while of about the same breadth as those of larger fruit bodies, are distinctly shorter. He has also shown† that the hairy scale cells on the pilei of fruit bodies of *Coprinus lagopus* are larger in large fruit bodies than in small fruit bodies. Table VIII shows the relation between the diameter of the pileus and the size of the spores produced for twenty-three fruit bodies of *C. sterquilinus*.

Table VIII.

Number of fruit bodies considered	Diameter of pileus in cm.	Mean length of spores in microns
2	1	15·5
2	5	16·8
5	6	18·3
4	7	18·4
9	8	18·2
1	9	18·3

From a consideration of the data set forth in Table VIII we may conclude: (1) that fruit bodies with small pilei produce small spores; (2) that the size of the spores increases with the diameter of the pileus until the latter reaches 7–8 cm. which is normal for the species; and (3) that a further increase in the diameter of the pileus is not correlated with any further increase in the size of the spores.

VII. Viability of Large and Small Spores.

It is evident from the data which have already been presented that spores of *C. sterquilinus* vary greatly in size. In the many spore deposits examined, the largest spore found measured 23·3 μ in length, while the smallest spore found measured 10·7 μ in length. The largest and smallest spores observed to germinate were respectively 22·8 μ and 12·6 μ in length. Extremely large or extremely small spores must be regarded, therefore, not as abnormal or imperfectly formed

* Buller, A. H. R. Researches on Fungi, II (1922), 86.
† Ibid. III (1924), fig. 141, p. 320.

structures, but as viable reproductive bodies capable of performing their normal functions.

As already pointed out in Section II, larger spores were found to germinate about twice as well as smaller ones. Hence we must conclude that small spores are less viable than large ones.

VIII. The General Range of Variation in Spore Size for the Species.

The extent of the variation in size of the spores of *C. sterquilinus* is shown by the frequency curve in Fig. 1. This curve was constructed from measurements of the lengths of a hundred

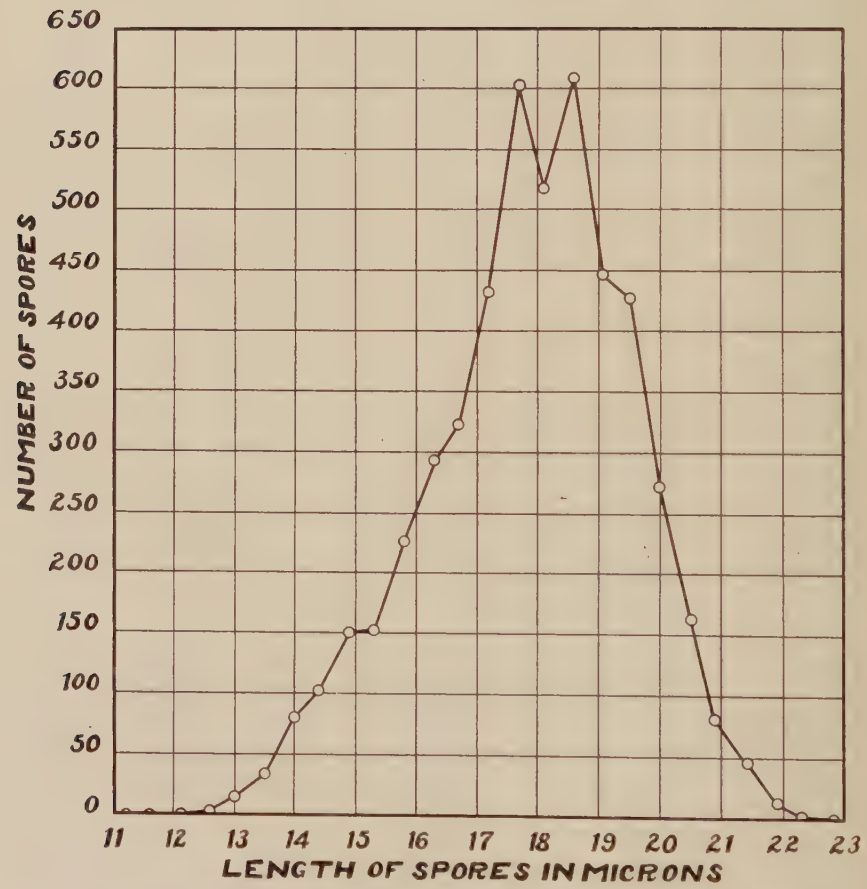

Fig. 1. Frequency distribution of spores from fifty fruit bodies of *Coprinus sterquilinus*, according to spore length. One hundred spores from each fruit body were measured. The class interval is 0·465 μ.

spores from each of fifty fruit bodies. As is shown graphically, the 5000 spores measured vary in length from 11·2 μ to 22·8 μ with a mean at 17·8 μ. Notwithstanding the large number of spores measured, the distribution is very irregular. This unevenness, undoubtedly, is due to the fact that the curve is multimodal in nature, with the spores from each fruit body, or from each group of similar fruit bodies, occupying a particular position on the curve.

Fig. 1 presents a general picture of the complete range of variation in spore size for the species, but gives little information as to the frequency distribution of the spores from the different types of fruit bodies which go to make up the compound curve. In view of the difficulty of showing frequency curves for a range of fruit bodies having modes for spore length from 14·4 μ to 20·5 μ, only three types of fruit bodies will be considered: (1) those having modes for spore length of 14·4 μ–15·3 μ; (2) those having modes for spore length of 15·8 μ–16·7 μ; and (3) those having modes for spore length of 20·0 μ–20·5 μ. The first group is made up of four fruit bodies, the second of three fruit bodies, and the third of three fruit bodies; and 100 spores from each fruit body were measured. The three types of frequency distribution are shown in Fig. 2. Two of the curves are asymetrical in form; the curve for spores from large-spored fruit bodies has a negative skew, while that for spores from small-spored fruit bodies has a positive skew. The distribution of the spores from fruit bodies having spores with modes for length from 15·8 μ–16·7 μ approaches in form the normal frequency curve. A measure of the skewness may be obtained from the formula $\frac{\text{mean mode}}{\sigma}$, where σ refers to the standard deviation. The coefficients of skewness, as calculated from this formula, are as follows:

Group 1 (Modes 14·4 μ–15·3 μ) = + 0·294
„ 2 (Modes 15·8 μ–16·7 μ) = 0
„ 3 (Modes 20·0 μ–20·5 μ) = − 0·557

In fruit bodies having modes for spore length of 14·4 μ–15·3 μ, the lower limit of spore size seems to have been reached. If smaller spores than these were to form on the gills of a fruit body of *C. sterquilinus*, they would probably not mature and, therefore, would not be shed by the fruit body. For this reason, a frequency curve for spores from such fruit bodies falls off rapidly on the lower side and gradually on the upper side, as is shown by the coefficient of skewness. In fruit bodies having modes for spore length of 15·8 μ–16·7 μ, a wide range of variation is possible both below and above the mode, so that spores from

such fruit bodies show a normal frequency distribution. In fruit bodies having modes for spore length of 20·0 μ–20·5 μ, the upper limit of spore size is approached and variation is possible only in the direction of smaller spores; a frequency distribution

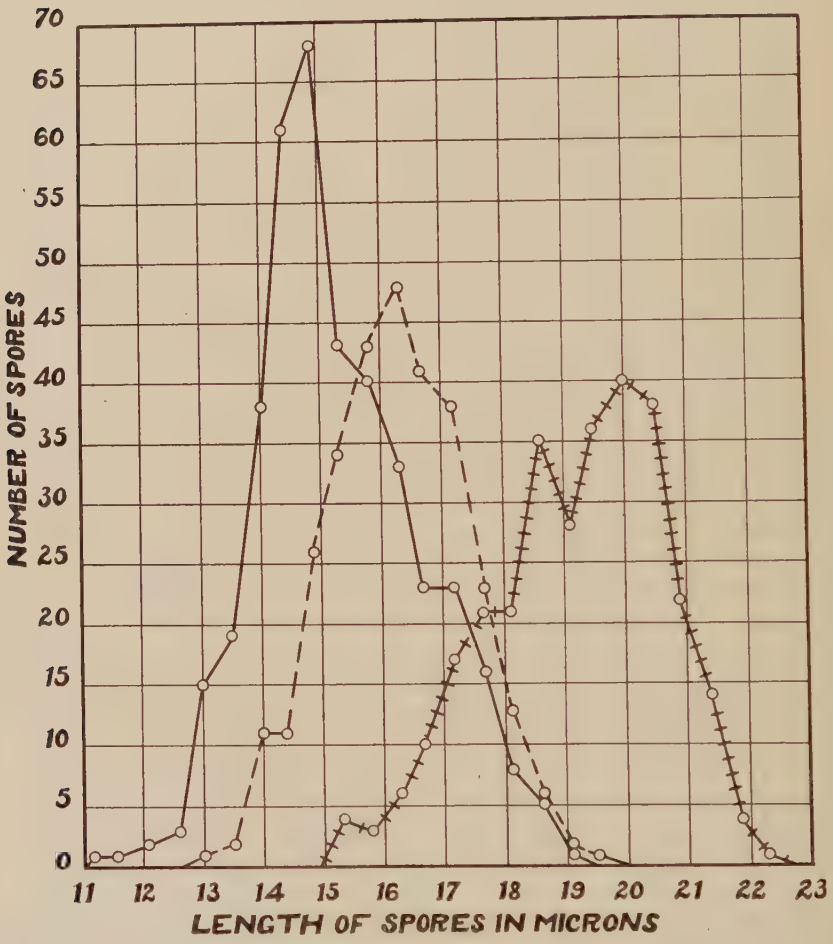

Fig. 2. Frequency distribution of spores from three groups of fruit bodies of *Coprinus sterquilinus*, according to spore length. Group 1, four fruit bodies with modes for spore length 14·4 μ–15·3 μ; group 2, three fruit bodies with modes for spore length 15·8 μ–16·7 μ; group 3, three fruit bodies with modes for spore length 20·0 μ–20·5 μ. One hundred spores from each fruit body were measured. The class interval is 0·465 μ.

for spores from such fruit bodies, therefore, shows negative skewness. If the fruit bodies which have been studied had

represented distinct races of *C. sterquilinus* differing from one another in respect to the size of their spores and breeding true for such a character, these distinct types of frequency distribution would not have been expected to occur. The nature of the variations in spore size in the fruit bodies studied, therefore, would seem to provide additional evidence that fruit bodies of *C. sterquilinus* which produce large spores do not differ genetically from those which produce small spores. Variations in spore size must be attributed rather to the particular physiological conditions under which the fruit bodies develop.

IX. Summary.

1. An experimental study has been made of the inheritance of spore size in *Coprinus sterquilinus*, one of the homothallic Hymenomycetes.

The character on which selection was based was length of spore. The original spore selections were made from six wild fruit bodies. In one series of experiments, each generation was propagated from the smallest spore which could be found; in another series of experiments, each generation was propagated from the largest spore which could be found. Selections from three fruit bodies were carried on for five generations, those from one fruit body for three generations, and those from two fruit bodies for two generations. Records were kept of the length of each mother spore, and of the mean length of the spores from the progeny fruit body of each monosporous mycelium. All spores were measured when *dry* with the Poynting Plate Micrometer.

2. The attempt to produce large-spored strains and small-spored strains in pure lines of *Coprinus sterquilinus* by the continuous selection of large and small spores respectively failed. No satisfactory evidence of the inheritance of individual variations in spore size was found. In this respect the spores of *Coprinus sterquilinus* resemble the seeds of the garden bean *Phaseolus vulgaris nana* investigated by Johannsen, the conidia of *Phytophthora infestans* investigated by Rosenbaum, and the spores of *Pestalozzia Guepini* and *Helminthosporium teres* investigated by La Rue.

3. There is considerable variation in the mean length of the spores produced by a fruit body at different times during the spore-discharge period.

4. Fruit bodies having small pilei produce smaller spores than those having pilei of the normal size or larger.

5. Since the mean size of the spores of different fruit bodies of one and the same species of Hymenomycete vary considerably, the spore size given by systematists for determining hymenomycetous species ought to be based on measurements of the spores of a number of fruit bodies obtained in different places.

6. The largest and smallest spores found were respectively 23·3 μ and 10·7 μ in length; the largest and smallest spores observed to germinate were respectively 22·8 μ and 12·6 μ in length.

7. The percentage germination of spores between 17·2 μ and 23·3 μ in length was 31·4; that of spores between 10·7 μ and 16·7 μ in length was only 16·2. Thus larger spores were found to germinate twice as well as smaller ones.

8. Frequency curves of spore length are presented for fruit bodies having modes for spore length of (1) 14·4 μ–15·3 μ, (2) 15·8 μ–16·7 μ, and (3) 20·0 μ–20·5 μ. The relation between the forms of these curves and the nature of the variation in spore size in fruit bodies of *Coprinus sterquilinus* is discussed.

9. Miss Baden's conclusions (1) that spores of *Coprinus sterquilinus* germinate only in the presence of certain bacteria and (2) that they are not ready to germinate when they are shed have not been confirmed.

The foregoing investigation was carried out in the Botanical Laboratory of the University of Manitoba during the tenure of a scholarship awarded by the Canadian Society of Technical Agriculturists; a grant in aid of this work was also made by the Canadian Honorary Advisory Council for Scientific and Industrial Research. The problem was suggested by Professor A. H. R. Buller, whose valuable advice and stimulating criticism is gratefully acknowledged.

RHACOPHYLLUS B. & BR.

(With Plate X and 2 Text-figs.)

By T. Petch, B.A., B.Sc.

In the *Fungi of Ceylon*, Berkeley and Broome instituted a new genus of Agaricaceae, *Rhacophyllus*, giving the generic description, "Pileus tenuissimus, tenerrimus; lamellae in fragmenta oblongo-obtusa flexuosa divisae." The single species, *Rhacophyllus lilacinus*, was described as follows:

"301 *R. lilacinus* B. & Br. (No. 825, *cum icone.*)

"On dead wood, twigs, etc. Peradeniya, Dec. 1868, Jan. 1869.

"Pileus cylindrical or digitaliform, lilac, striate, or even[,] split more or less at the margin; stem dilated at the base, attenuated upwards; gills replaced by numberless oblong, irregular, waved, obtuse lobes of the same colour as the pileus.

"It is possible that there may be two species; but the number of specimens gathered at present is very small. *Pterophyllum*,

Mont., agrees somewhat in character; but it is closely allied to *Panus*, while this is more closely to *Coprinus*."

Berkeley and Broome's description was drawn up chiefly from the paintings sent by Thwaites. The figures were reproduced in *Journ. Linn. Soc.* XIV (1873), plate 2. Figure *s* which Berkeley and Broome described as "gills, with processes more highly magnified" is marked by Thwaites on the original, "Shred from the pileus with laminae," which, on examination of the specimens, is perceived to be a very different statement from that of Berkeley and Broome.

Fig. 1. *Rhacophyllus lilacinus*, nat. size.

On Thwaites's second sheet of figures, he marked one, "probably something else." This is a group of three contiguous pilei, sessile on the substratum, the stalks not having yet expanded. The colour is brownish white, different from that of the mature specimens, and it was probably for this reason that Thwaites suggested that they might be a different species. This figure is reproduced in *Journ. Linn. Soc.* (*loc. cit.*), as figure *e* (left), but one would hardly realise from the reproduction that the original shows three distinct cylindric pilei, acute at the apex. Berkeley and Broome's suggestion that there were two species in the type was evidently a repetition of Thwaites's note. These immature specimens are not in Herb. Peradeniya. It would seem probable from the figure that they were merely immature examples of *Rhacophyllus lilacinus*.

Rhacophyllus has been regarded as an agaric damaged by insects or as an abnormality. F. Ludwig, in 1908, in a paper on abnormal fructifications in Basidiomycetae, suggested that *Rhacophyllus* was a polyporoid *Coprinus*, *i.e.* a *Coprinus* in which the lamellae were replaced by pores, but it is evident that he had not seen a specimen.

In 1901 Patouillard described similar specimens from Tunis. These grew on rotten tree trunks, arising in groups from a white mycelium. The fully-developed fungus had a campanulate, greyish fawn pileus, 8–10 mm. high, striate and deeply laciniate at the margin, and a fragile, white, cylindric, fistulose stalk, up to 2 cm. high and 1·5 mm. diameter. The gills were rose purple, some reaching the apex of the stalk, and others shorter.

The trama of the pileus was reduced to a very thin membrane. The gills consisted of series of small fleshy particles, flattened, approximately of the same size, united by their edges, adhering only feebly to one another and readily separating. The substratum was usually powdered with these fragments of the gills.

These particles were irregularly orbicular, from 150 to 300 μ

diameter, 70–100 μ thick, thinning out towards the edge and somewhat biconvex. They consisted entirely of angular cells, 10 × 6 μ, hyaline, thin-walled, with refringent contents. The contour of these cellular masses was well defined. A few irregular filaments were observed, binding them to one another, or they were united by their edges in groups of three or four.

In the earlier stages, the fructification (receptacle) was entirely enclosed in a universal veil which soon disappeared. The margin of the pileus was straight and in contact with the stalk.

Transverse sections showed that in young specimens the gills were continuous, but in slightly older specimens the formation of the cellular masses had already begun and they appeared as compact, white islets, arranged in rows, united by delicate hyphae which gradually disappeared as the fungus developed.

Patouillard regarded these structures as bulbils. Instead of gills, the fungus produces sheets of biconvex bulbils, loosely united by their edges.

The hyphae of the bulbils were at first cylindric, short, and continuous. Subsequently they became torulose, transversely septate; and finally were divided into angular cells which formed a compact pseudoparenchyma.

Patouillard stated that this production of bulbils occurred at all points where normally basidia would be formed, and thus involved the interlamellar spaces as well as the gills. The entire thickness of the gills was transformed.

No normal agarics were obtained in company with the Tunisian examples. Similar specimens were received by Patouillard from Guadeloupe, differing only in that the pileus was more conical than in the Tunisian examples, and that they grew in tufts instead of being scattered. The specimens from Guadeloupe, however, were accompanied by a single example, which the collector (Duss) had noted as being the normal state of the fungus. The latter was a *Psathyra*, near *Psathyra gyroflexa*. Consequently Patouillard considered that the forms in which the gills were converted into bulbils were probably derived from a *Psathyra* or *Psathyrella*, taking into account their general shape and aspect.

In 1913, Patouillard described similar specimens from Tonkin, and assigned them to *Rhacophyllus*. The Tonkin specimens were very small, only 2 mm. high, globose before expansion with a regularly cylindrical stalk which arose from a small disc. The bulbils were pyriform, attached to the interior surface of the cap by the broader end, with the free acute tip directed towards the centre; they were 0·25 to 0·3 mm. long, and were arranged side by side in a single row. The specimens from Tunis and Guadeloupe differed from those from Tonkin in having the

bulbils smaller and more rounded, and arranged in five or six rows.

Patouillard was of opinion that these bulbiferous forms were derived from different agarics, in all probability related to *Psathyrella*.

Berkeley and Broome's reference to *Pterophyllus* was considered by Patouillard, who states that *Pterophyllus Bovei* is a true *Pleurotus*, and that the masses on the faces of the gills are superficial accumulations of spores.

The bulbils of Patouillard's specimens (preserved in alcohol) were studied by Moreau, who states that the majority of the cells contain two nuclei, the normal number in the cells of the pileus of a Basidiomycete. The two nuclei approach one another and fuse, so that the cells become uninucleate. The nucleus then undergoes mitosis, presenting a spindle with two chromosomes and two centrosomes, a typical mitosis of a Basidiomycete. In consequence of this mitosis, the primitive number of nuclei in the cells is restored. Each nucleus then divides again, so that the cells become quadrinucleate.

So far, the nuclear history of the cell comprises a karyokinesis, followed by a chromosome reduction and two successive mitoses. This is exactly the same as occurs in basidia. Each cell of a bulbil, from the point of view of its nuclear history, is homologous with a basidium.

Two of the four nuclei degenerate, and thus the primitive number of nuclei is again restored. Sometimes a supplementary mitosis occurs and six nuclei are found in a cell.

The foregoing details concerning the bulbils are all taken from Moreau's account.

Moreau also states that a perforation sometimes appears in the wall between two adjacent cells, and the protoplasm of the two cells unites.

Cultures.

In July, 1916, specimens of *Rhacophyllus lilacinus* were found at Henaratgoda, growing on a recently felled log of *Ficus nervosa*. Parts of the log were cut off and conveyed to Peradeniya, where they were kept in a sink in the laboratory and periodically watered. Some further specimens developed in transit, and a succession of others appeared until the middle of September. The specimens were scattered or clustered, and there was no superficial mycelium. No normal agarics appeared on the wood, which was in two pieces, weighing together half a hundredweight. As the fructifications matured, the bulbils fell and formed small patches round the base of the stalk.

Bulbils were sown on blocks of *Ficus* wood in Roux tubes on

September 4th. The blocks were cut from a newly felled branch, boiled (weighted down), fixed in the tubes with a plug of cotton wool at one side, and then sterilised three times. Good growth occurred at once, a white mycelium spreading over the wood and extending along the sides of the tube. The initial stages of the sporophores were observed on September 14th, and the sporophores were fully developed in one tube on September 16th. Four tubes out of six contained fully-developed sporophores on September 17th, while in the other two immature sporophores were present. The mycelium was somewhat feathery, with radiating strands. Up to fifty sporophores in various stages of development were present in a single tube, while over a dozen were expanded at the same time, filling the bore of the tube. Sporophores developed on the cotton wool and on the strands on the sides of the tube. They did not exceed one centimetre in height.

In one Roux tube from which the cotton wool plug had been omitted, the superficial mycelium was scanty, but sporophores were nevertheless developing on September 17th.

Bulbils were also sown on similar blocks resting on wet cotton wool plugs in boiling tubes, after sterilisation as before. In this series, the wood was more saturated with water than in the foregoing. Of six tubes started on September 12th, growth of mycelium was evident on September 17th only in two tubes which were drier than the others. On September 19th, growth was evident in three. On September 21st, the first two were producing fructifications; two others showed mycelium only; while nothing was evident in the two wettest. On September 26th, fructifications were present in four tubes, but no growth had occurred in the two wettest tubes. The experiments were then brought to a conclusion by the unexpected transfer of the writer to administrative duties.

In no case did any normal agarics occur in these cultures, nor did any appear on the original *Ficus* blocks.

Rhacophyllus lilacinus would appear to prefer a porous, well-aerated wood as a substratum. The wood of *Ficus nervosa*, and of the *Ficus* used in the above experiments, is very porous. It will be noted that the only failures were in tubes in which the wood was wetter than in the others.

Bulbils placed in hanging drops of water rapidly produced a mycelium. The hyphae arose chiefly from the marginal cells of the bulbil, and radiated outwards over the surface of the cover glass. Lateral branches were produced, those from adjacent radial hyphae fusing with one another and forming cross branches, so that the whole mycelium had the appearance of a cobweb.

Thus *Rhacophyllus lilacinus* can be reproduced from the bulbils, and all the examples obtained in that way have been identical in structure with the parent fungus. The enforced termination of the experiments prevented trials on other media.

Description of the Fungus.

The fungus is usually cylindric at first, rounded or pointed at the apex, sometimes ovoid, or sometimes in small examples globose. It is enclosed in a very thin universal veil, which disappears, or is represented by a few shreds on the pileus, when the fungus expands in nature, but may partly persist as a small shallow cup at the base of the stalk in examples grown in culture. The unexpanded fungus has the appearance of an immature *Psathyrella* or *Coprinus*, and its expansion consists chiefly of a lengthening of the stalk.

The expanded fungus is up to 3 cm. high, but the majority of specimens do not exceed half that height. The pileus is up to 1 cm. high, conical, or conico-cylindric, or conico-campanulate, with a rounded apex, purple-rose, feebly striate, lacerate at the margin. The stalk is white, up to 3 cm. high, smooth, expanded at the base, attenuated upwards, up to 1 mm. diameter in the middle, arising from a flat tomentose disc. The fructifications may be either scattered or clustered. In nature, in the specimens hitherto seen, they do not arise from an evident mycelium, but in culture they may spring directly from the wood or from strands of mycelium which spread over the wood and the sides of the tube.

In the positions usually occupied by the gills of an agaric, the fungus bears sheets of more or less flat "bulbils," arranged edge to edge in the same plane. The plane which may be considered to represent one gill may contain over thirty of these bulbils, varying enormously in size and shape. In general, they are irregularly angular with rounded corners, sometimes long triangular, sometimes lobed, sometimes evidently composed of two or more fused together by their narrow edges. They are up to 40 μ thick, and vary in size from 0·3 × 0·2 to 1 × 0·4 mm. In the unexpanded fungus, the plane of a single gill shows some bulbils extending from the tissue of the cap to the stalk, and others in a row of two or three across the gill. Some are united to the cap, others to the stalk, while others are free from either. The bulbils which occupy the position of a single gill are not, as a rule, in contact with one another at their edges, but are separated by spaces, 4–16 μ broad, which contain a weft of hyphae. The bulbils are not quite flat, but, as noted by Berkeley and Broome, somewhat undulating. They are not biconvex. They are pale purple-rose when mature, and to them the

apparent colour of the pileus is due. When dry they are pale purple-brown.

The pileus expands to an angle of about 20° on each side of the vertical. The bulbils then fall out, leaving a thin, hyaline, membranous cap. The tissue of the cap is about 0·1 mm. thick at the apex, diminishing to 20–25 μ at the lower edge, and is composed of radial, parallel, septate hyphae, with segments varying from 4 to 8 μ in diameter.

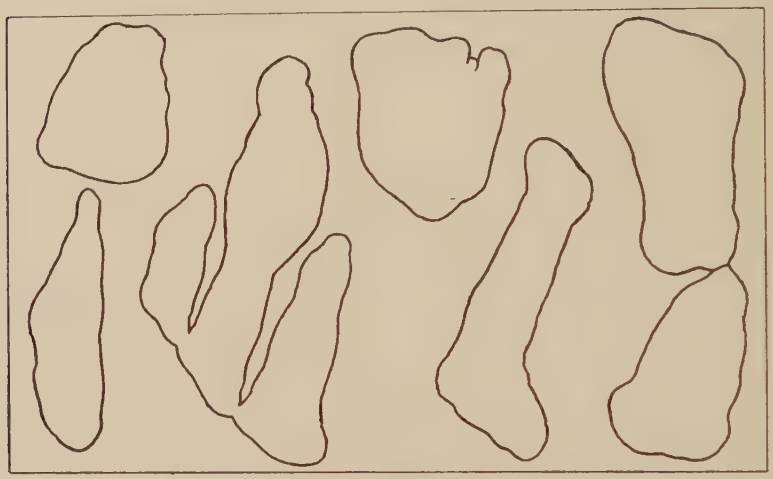

Fig. 2. Bulbils of *Rhacophyllus*, ×150.

The bulbils are of almost uniform thickness, with rounded edges, and are composed of thick walled, polygonal cells. At the narrow edge, there may be a layer of parallel hyphae, two to four hyphae thick, but in general only the disorganised remains of these hyphae persist, with sometimes a short, free fragment of a hypha extending from the bulbil. This disorganised layer does not cover the flat sides of the bulbil, and there is no differentiated cortex. Under pressure, most of the cells of the bulbil readily separate. The individual cells are more or less hexagonal, 6–9 μ diameter, or angularly ovoid, up to 12 × 8 μ, with a wall about 2 μ thick. Some remain united in short chains of two to five, or in plates of six or eight. Some cells bear a thin solid projection up to 6 μ long from one angle; others may have two, from adjacent angles. These projections lie between the walls of the adjacent cells. It is not clear what they represent.

Although the bulbils are composed of thick walled cells, they can scarcely be styled sclerotia. The cells are arranged more or

less in rows from one side of the bulbil to the other. The bulbil is not formed from irregularly intertwined hyphae, as are sclerotia in general. Nevertheless, they are sclerotioid in nature, though not in mode of formation.

A longitudinal section, almost median, of an example just prior to expansion is given on Plate X, fig. 1. Owing to the curvature of the stalk, the latter and the bulbils are cut obliquely. As the bulbils do not lie exactly in radial planes, and as they are usually curved, it is difficult to obtain a section showing a complete radial series, but the left-hand side of the figure shows one approximately. The annular appearance of the bulbil near the apex is due to the fact that it was concave and the central portion has been cut off. Similarly, the sickle-shaped bulbil a short distance below the former is only the lower part of one. At the lower right-hand corner, the planes of the radial series are twisted, and the bulbils are cut transversely. This example was embedded in celloidin, and the hyphae which run between the bulbils are not visible.

Cross-sections of the developing fructification show that it is solid (Plate X, fig. 2), the plate-like bulbils being embedded in a plectenchyma which fills all the spaces between them. There are no interlamellar cavities. The plectenchyma descends to the outer edge of the pileus, the clefts in the margin of the latter being continued inwards for a short distance. When the stalk lengthens and the pileus expands, the hyphae of the plectenchyma are torn apart and the bulbils fall out. Some of the hyphae persist, forming loose strands extending from the cap (Plate X, fig. 7).

Of the rows of bulbils seen in a cross-section, some extend from the pileus to the stalk, others are in contact with one or the other, while others are not in contact with either. It is, however, obvious, that as the bulbils do not form a continuous tissue in a vertical gill plane, the number which appear to be attached to the pileus or the stalk will vary at different heights, especially as the edge of a bulbil is frequently curved. But it is also clear that there are broad and narrow planes of bulbils, corresponding with the long and short gills of an agaric. In one instance, of fifty-five radial lines of bulbils in the cross-section, twenty-six were attached to the stalk, and forty-eight to the pileus. Sometimes the bulbil appears to be merely in contact with the stalk or the pileus. In other cases it is attached by a narrow zone of parenchyma, the cells of which are smaller than those of the pileus or stalk respectively.

To make this account clearer, we may provisionally accept the view that the rows of bulbils are homologous with the lamellae of an agaric. For convenience, the bulbils which lie in the same

radial vertical plane will be referred to as a lamellar series, or as a lamella.

The youngest example examined was 0·8 mm. high, and 0·5 mm. diameter. Its height was probably abnormal for the stage of development attained, owing to the fact that it arose partly beneath the base of a larger specimen. In this, the stalk primordium was well developed, and composed of rather large longitudinal hyphae. The outline of the pileus was evident as a zone, convex above, but the pileus was not separated from the universal veil. In a tangential section, a short distance from the median plane, the tissue above the stalk primordium was 100 μ thick. Of this, the thickness of the universal veil was 50 μ, the latter being distinguished from the pileus primordium by its larger irregular cells. From the pileus primordium, a series of vertical, rather distant trabeculae extended downwards into the fundamental tissue; these, like the pileus primordium, stained more deeply with haematoxylin than the surrounding fundamental tissue. These trabeculae are the primordia of the lamellae. There is no annular gill cavity. The total depth of the zone occupied by the primordia of the lamellae and the pileus was 50 μ.

In globose specimens 1 mm. diameter (Plate X, figs. 3, 4) the stalk was clearly outlined, and occupied the greater part of the developing fungus. It was broadly conical, composed of vertical hyphae, the outer hyphae being (in part at least) continuous with the fundamental tissue. The pileus was well developed, its radial structure being clearly evident. The tissue between the pileus and the stalk formed, in longitudinal section, a narrow segment, almost the whole length of the stalk, and 0·25 mm. broad at the widest part. The bulbils were in process of development in this fundamental tissue. They arise at different points in the same vertical plane (Plate X, fig. 4). They do not originate in a continuous sheet which divides into bulbils later. In longitudinal sections along the plane of a bulbil, the cells of the latter appear rather loose at first. In sections, especially transverse sections of the fungus, which cut a bulbil at right angles, it is seen that many of them are hollow, and others show a line of division down the middle (Plate X, fig. 5). In the hollow bulbils, a palisade layer of short cells lines the cavity, while the others consist of two layers of palisade cells, in contact and united at their margins. Some developing bulbils are partly hollow and partly solid.

The bulbils are formed by short branches perpendicular to the vertical hyphae of the fundamental tissue. These branches arise close together, forming a short palisade tissue, completely enclosing a small area in a vertical radial plane. All the

branches are directed inwards towards that area. Consequently there is formed a layer of palisade tissue round a narrow cavity, or more usually a plate of tissue consisting of two opposed palisade layers, which merge into one another at their edges (Plate X, fig. 6). The cells of the palisade layer produce cuboid or oval, spore-like cells, 2–3 μ diameter, at their apices, *i.e.* within the developing bulbil; these cells do not become free, but remain united in short chains, which, at least in the early stages, lie in line with their parent cells.

It would be expected that the bulbils arise in radial vertical planes of primordial tissue, in continuation of the more deeply staining primordia which descend into the fundamental tissue from the pileus primordium in the earlier stages of development. But when bulbil formation has begun, no deeply staining strands have been detected between the bulbils of a single lamellar series. Moreover, the fact that the bulbils are discontinuous in a lamellar series argues against the existence of a lamellar primordium continuous from the pileus downwards.

No longitudinal hyphae are present within the bulbils. They do not include any of the hyphae of the fundamental tissue. Consequently it would appear that the opposed horizontal branches arise from hyphae which lie close together.

The presence or absence of a cavity within the bulbil appears to depend merely on the number of palisade branches which arise round a given centre. From the general shape of the bulbils, the prior existence of cavities round which they might be formed appears improbable, and such cavities have not been observed. That the cavities in the bulbils are not artefacts would seem to be clear from their general shape and structure. The material was taken from culture and fixed in chrom-acetic acid. In any case, whether the cavity is artificial or not, it is quite clear that the bulbils are formed from without inwards.

There is no general curvature of the hyphae of the fundamental tissue into the bulbil. Some do curve towards the bulbil, but there is nothing resembling the general curvature of hyphae towards the hymenium seen in the trama of a developing gill.

Retaining the comparison with an agaric, we may visualise the development of *Rhacophyllus* as follows. In a normal agaric, the gill primordia develop into plates of tissue which produce a palisade layer of basidia on each side, *i.e.* externally, relatively to the primordial tissue. On the other hand, in *Rhacophyllus* the hypothetical gill primordium develops a palisade tissue internally from either side, and these cells unite into a solid tissue.

The bulbils increase in size, and, if hollow, become solid, by the addition of cells internally from the apices of the original

palisade cells, and by the enlargement of the individual cells. It is possible that in an early stage new branches from the fundamental tissue may penetrate between the cells of the original palisade layer. But they do not show any indication of the addition of cells externally.

With reference to the formation of bulbils discontinuously in the fundamental tissue, it is of interest to note that the hyphae of the fundamental tissue may form chains of spore-like cells at any point. In sections of the mature fungus, it is not uncommon to find spore-like cells lying in the interlamellar tissue. These, however, are best seen in sections of a specimen from which some of the bulbils have fallen (Plate X, fig. 7). The interlamellar hyphae which then extend from the pileus or stalk may bear short chains of thin-walled cells, either cuboid, about 3 μ diameter, or oval, 3 × 2 μ. These arise laterally from the hyphae, and the chains may branch and form small clusters. But that the formation of the bulbils is not due to a haphazard production of chains of cells is evident from their occurrence in definite lamellar series.

The development of *Rhacophyllus* differs from that of the majority of the agarics which have been studied, in the absence of an annular gill cavity or furrow. But in this respect it agrees with *Amanitopsis vaginata* as described by Atkinson. In the latter there is no gill cavity, the primordia of the lamellae appearing first as trabeculae embedded in the fundamental tissue. In its further development, *Rhacophyllus* differs completely from *Amanitopsis vaginata*, as no interlamellar spaces are formed, and bulbils are produced instead of gills.

Rhacophyllus has been regarded as an agaric, in which the gills have been converted into bulbils. It will be evident that such a generalisation does not convey a correct impression. *Rhacophyllus* does not possess true gills, and the structures which occupy the position of the gills of an agaric are formed in a manner which may be said to be the reverse of that of a gill. In the latter respect, they agree more closely with the formation of the hymenium in the Lycoperdineae.

Systematic.

The systematic position of *Rhacophyllus* is doubtful. In the possession of a universal veil, a definite radial pileus, and a stalk, composed of parallel hyphae, which lengthens at maturity, it is decidedly agaricoid; and its general appearance, both before and after expansion, is that of a *Psathyrella* or *Coprinus*. But none of these features is confined to the Agaricaceae. *Battarrea Steveni* (Tulostomataceae), as described by de Bary, has a universal veil, and its stalk, scarcely evident in the un-

expanded fungus, attains a length of 1 ft. on expansion. *Polyplocium* and *Gyrophragmium* (Hymenogastrineae) have a central stalk, which elongates when the fungus is mature, and a volva; but whether the latter represents the remains of a universal veil or not does not appear to have been ascertained.

Another genus of the Hymenogastrineae, *Secotium*, has a similar stalk in some species. *Secotium agaricoides* (Czern.) Holl. has been recently studied by Conard, who found that the developing fungus possesses an outer layer homologous with the universal veil of an agaric, and is furnished with a gill cavity. The hymenium is borne on branched and folded anastomosing lamellae which grow downwards into the gill cavity, just as in the case of *Agaricus*, except that the lamellae are not plane. Consequently, Conard considers that *Secotium agaricoides* should be placed in the Agaricaceae, and that his results strengthen Fischer's opinion that the Secotiaceae should be dismembered and the genera distributed amongst the Hymenomycetes.

In *Secotium Mattirolianus* (Cav.) Fisch., the gleba in the unexpanded fungus does not show any lamellar arrangement in either longitudinal or cross section (cf. Fischer, in Engler-Prantl); it consists of branching and anastomosing folds, so that a section shows irregular cavities without any definite arrangement. *Secotium agaricoides*, according to Conard's photographs, presents the same appearance. Hence, if these can be included in the Agaricaceae, there would be little hesitation in similarly including *Rhacophyllus*, which has its bulbils in definite lamellar series, were it not for the structure of the bulbils.

The fact that the bulbils originate within the fundamental tissue, *i.e.* without the prior formation of a gill cavity, would not exclude *Rhacophyllus* from the Agaricaceae, since a similar condition occurs in *Amanitopsis vaginata*. But the mode of formation of the bulbils is opposed to any such reference.

If *Rhacophyllus* is considered to be derived from one of the Basidiomycetae, it can be compared, as regards the formation of the bulbils, only with species in which the basidia are formed as a lining to a small regular closed cavity. That rules out the Hysterangiaceae, in which the chambers of the gleba are formed by the fusion of trama plates, and most of, if not all, the Hymenogastraceae. It would appear that such a formation can be paralleled only on the Lycoperdineae or the Nidularineae, or, possibly, in some of the genera of the Plectobasidineae. But the development of the majority of the species of the last-named group has not been investigated, and it is not certain that true hymenial cavities occur in any of them. As regards the presence of a stalk, it would be paralleled in that group by *Podaxon*.

In the absence of spores, it is perhaps natural to regard *Rhacophyllus* as an abnormal condition of some sporiferous fungus. But there is no evidence in support of that supposition, unless we accept the single specimen of a *Psathyra* which Duss forwarded with his specimens of *Rhacophyllus* from Guadeloupe, and in that instance there is nothing to indicate why Duss considered the two related. No normal agarics occurred with the Ceylon specimens, nor did any develop later from the material brought to the laboratory or in culture. *Rhacophyllus lilacinus* can be reproduced by means of the bulbils, and the latter are sclerotioid and consequently capable of surviving conditions adverse to growth. There is therefore no sufficient reason why *Rhacophyllus* should not be an autonomous fungus.

It would appear probable, from the description, that the specimens from Tonkin described by Patouillard were *Rhacophyllus lilacinus*. The specimens from Tunis, according to the description and figures, would appear to be another species, differing from the former in the shape of the bulbils.

No attempt has been made to repeat Moreau's cytological investigations. Material fixed in chrom-acetic acid is available.

I am indebted to Mr L. S. Bertus for the slides and photographs.

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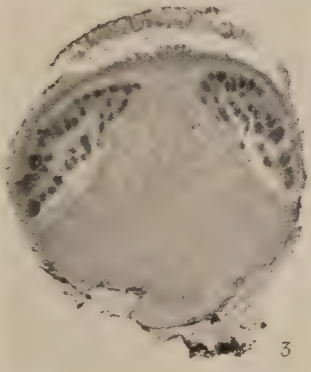
EXPLANATION OF PLATE X.

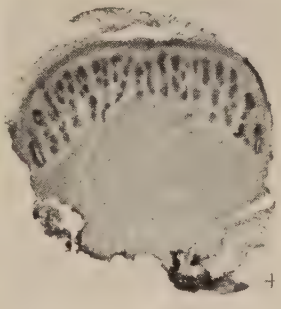
Fig. 1. Longitudinal section of a fructification just prior to expansion. ×15.

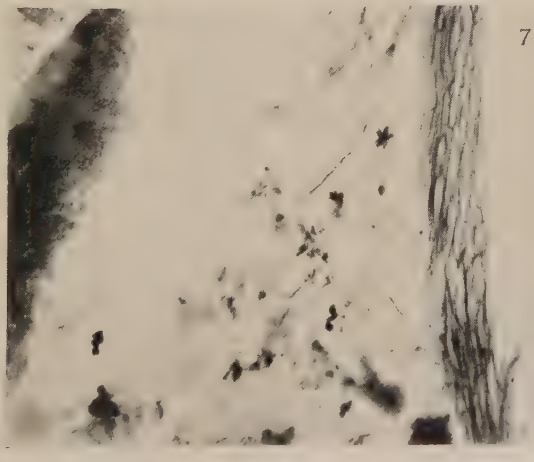
Fig. 2. Cross section of a fructification, 5 mm. high, shortly before expansion; the universal veil partly separated from the cap. ×40.

Fig. 3. Median longitudinal section of a developing specimen, 1 mm. diameter, part of the universal veil detached in sectioning. ×40.

Fig. 4. Tangential section of the same specimen as in fig. 3. ×40.







RHACOPHYLLUS LILACINUS

Fig. 5. Cross section of an immature specimen, cut obliquely. ×40.
Fig. 6. Part of the section in Fig. 5. ×200.
Fig. 7. Part of a longitudinal section of a mature specimen from which some of the bulbils have fallen. ×300. On the right, section of the cap, to which are attached strands of hyphae which bear chains of cubical cells in groups; on the left, part of a bulbil cut obliquely.

STUDIES IN ENTOMOGENOUS FUNGI.

(With 1 Text-fig.)

X. VERTICILLIUM SPP.

By T. Petch, B.A., B.Sc.

A SPECIES of *Verticillium* parasitic on *Aleyrodes* on *Citrus* was collected in Florida in 1905, and has since been found to be generally distributed in that state. It is not confined to *Aleyrodes*, but has occurred also on *Mytilaspis*, *Diaspis*, and *Lecanium*. The fungus was studied by Fawcett, who published a full account of it in his *Fungi parasitic upon Aleyrodes Citri* under the name *Verticillium heterocladum* Penzig. I have specimens of this species from Fawcett, and others from Webber, the latter in association with *Aegerita Webberi*. The first specimens recognised were also in association with *Aegerita Webberi*, but Fawcett has shown that the two are quite distinct.

The fungus is peculiar, as a *Verticillium*, in forming superficial stromata. In this respect it resembles *Aegerita Webberi*. The stromata are cinnamon brown, becoming ashy brown, flattened pulvinate, up to 2 mm. diameter, and 0·6 mm. thick. The surface of the mature stromata appears powdery, and when magnified is seen to be covered with free ends of hyphae. The stromata is compact, not spongy, and brownish yellow internally. There is usually a cavity at the base, up to 0·2 mm. high, in which the insect is situated. Each stroma covers a single insect, and, as in the Florida specimens of *Aegerita*, the scale persists beneath the stroma.

The hyphae of the stroma are 2–5 μ diameter, brownish yellow in the interior of the stroma, but hyaline towards the periphery. They are contorted and intertwined, but not fused into a parenchymatous tissue. The walls of the hyphae are thickened, so that the lumen is only about 1 μ diameter, but no completely solid hyphae have been observed. The septa persist, unthickened, in these thick-walled hyphae, and appear as narrow lines across the slender lumen. The contents of the hyphae stain with eosin, but the walls do not. As noted by Fawcett, the hyphae of the stroma tend to break up into short spore-like lengths.

The stromata are bordered by a rather coarse, byssoid or strigose margin, or hypothallus, about 1 mm. broad, which merges into a thin, whitish, byssoid or pulverulent film, extending indefinitely over the leaf. The hyphae of the hypothallus and the film are regular, hyaline, 2–4 μ diameter, with a few stouter, brown, 4–6 μ diameter. These hyphae are septate, and their walls are only slightly thickened. They sometimes bear sessile, oval, spore-like bodies, 6 × 4 μ, laterally, each just below a septum.

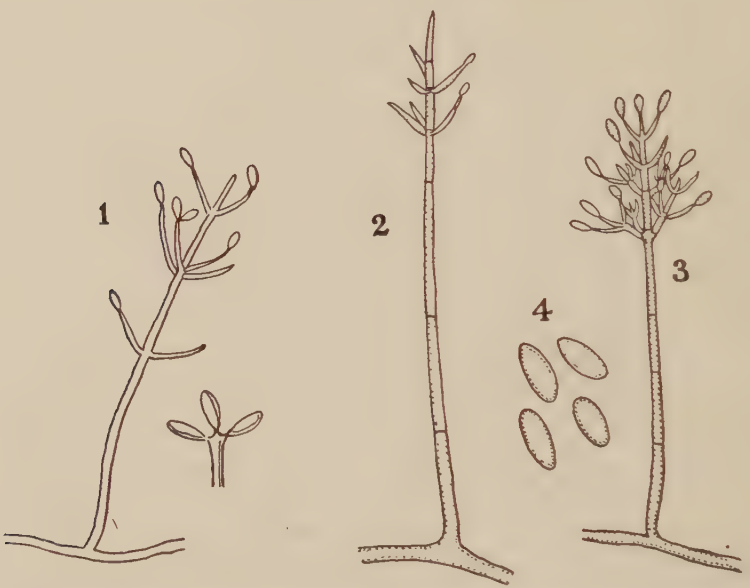


Fig. 1. *Verticillium heterocladum* Penz. 1, figure from *Fungi Italici* (magnification not stated); 2, young conidiophore, ×450 (Fawcett); 3, mature conidiophore, ×450 (Fawcett); 4, conidia, ×1000 (Fawcett).

The conidiophores arise from the hyphae of the film, or on the stromata. They are hyaline, erect, slender, septate, up to 240 μ high (Fawcett), and 2–3 μ diameter, with the basal segment sometimes inflated. At a considerable distance from the base, they bear either whorls of phialides, or whorls of branches which bear phialides at their apices. Usually there are two to four phialides or branches at a node. The lateral branches are more or less cylindrical, 6–9 μ long and 2 μ diameter. The phialides are elongated oval, attenuated above, somewhat inequilateral, curving upwards, 6–12 μ long, 2 μ diameter. The conidia are solitary, narrow oval, hyaline, and continuous. In

the specimens examined by me, they measured 3·5–4 × 1·5–2 μ. Fawcett gives the dimensions 4–6 × 1·5–2·5 μ.

Fawcett grew the fungus in pure culture on 5 per cent. glucose agar. The mycelium was at first white, with a reddish brown centre, ultimately becoming cinnamon-coloured and forming stromata. Conidia were produced at an early stage. In transfers to sweet potato and bread, the substratum became covered by a felted cinnamon-coloured stroma, while on rice and caladium stems the colours were ochre and brick-red respectively. The parasitism of the fungus was demonstrated by infecting larvae of *Aleyrodes citri* with spores from a pure culture.

This fungus has become generally known as the "cinnamon fungus." It has been recorded from the West Indies.

Fawcett referred this species to *Verticillium heterocladum* Penzig, a fungus which was originally found on *Lecanium hesperidum* on lemon leaves in Italy. It seems scarcely possible, however, that the identification can be correct. The original description of Penzig's species (*Michelia*, II, 462) is as follows:

Verticillium heterocladum Penzig. Hyphis repentibus, elongatis, paullum ramosis, continuis; ramis fertilibus adscendentibus, ramulosis; ramulis ternis vel quaternis, oppositis vel alternis, patentibus, rectis, apice attenuatis; conidiis in ramulorum apice solitariis, geminatis vel ternis, saepius pedicellis brevissimis suffultis, oblongis, hyalinis, 5·5–6 micr. long., 2–3 micr. crassis.

It is to be noted that Penzig made no reference to any stroma, and that he stated that one to three conidia might occur at the apex of a phialide. Except for those points, the description might well be taken to fit the Florida species.

Penzig's figures were reproduced in Saccardo, *Fungi Italici*, Fig. 1193. These do not show anything resembling the characteristic stromata of the Florida species. The fungus covers the scale and forms a narrow white border round it, resembling, in that respect, *Cephalosporium Lecanii* and *Acrostalagmus coccidicola*. The figure of the conidiophore resembles that of the Florida species in the shape and position of the phialides, but in all cases the phialides are shown arising in whorls from the main stem, not from whorls of lateral branches. A more serious difference, however, is in the mode of attachment of the conidia. In the figure of the conidiophores the basidia are acute at the apex, with from one to three conidia, but in the enlarged figure of a phialide the apex is obtuse and three-toothed, each tooth bearing a single conidium.

One might suppose that the latter figure was based on an error of observation. But in the original description and in

Studi botanici sugli Agrumi e sulle Piante affini (1887), p. 398, Penzig added an explanatory note in which he stated that the distinctive character of the species was the marked variability of the arrangement of the fertile branches (*i.e.* phialides); sometimes these were verticillate in threes or fours, sometimes opposite, or sometimes solitary and alternate; in the ternate attachment (inserzione) of the conidia the species approached *Cladobotryum* Corda, and it ought perhaps to be separated from *Verticillium* on that character. Thus Penzig emphasised the mode of insertion of the conidia which he figured, and consequently it would appear that he could scarcely have made a mistake in his drawing.

Penzig stated that it was often found associated, on leaves of *Citrus*, with the species which he assigned to *Acrostalagmus albus* Preuss, from which it was distinguished by its mycelium and its external appearance.

In *Bull. Soc. Myc. France*, XXVII (1911), 486, Fron recorded *Verticillium heterocladum* Penzig on chrysalides of *Cochylis ambiguella* in France. He stated that it was easily cultivated and showed a characteristic arrangement of the hyphae. The hyphae bore numerous branches, sometimes isolated, sometimes three to five in a whorl. His figures show a striking resemblance to *Cephalosporium* (*Acrostalagmus*). That of the general habit of the fungus would quite well represent the repent hyphae of *Cephalosporium*, while the conidiophores show exactly the arrangement and variation in number of the phialides which obtains in the entomogenous species of *Cephalosporium*. Only a single conidium is shown at the apex of each phialide.

Fron did not give particulars of his cultures, and it would appear that he did not observe colour changes such as those recorded by Fawcett. Moreover, his figures do not agree with the American species, nor does his fungus agree with that described by Penzig, who laid special emphasis on the occurrence of one to three conidia at the apex of each phialide.

I have not been able to examine European specimens of *Verticillium heterocladum*, and consequently cannot carry this investigation further.

STUDIES IN ENTOMOGENOUS FUNGI.

(With 1 Text-fig.)

XI. EMPUSA LECANII ZIMM.

By T. Petch, B.A., B.Sc.

In "De dierlijke Vijanden der Koffiecultuur op Java," Deel II (*Meded. uit 's Lands Plantentuin*, XLIV, 1901), Zimmermann described (pp. 25–27) a fungus, *Empusa Lecanii*, which attacked

the green bug (*Lecanium viride*) in Java. He styled it the "Black Scale-insect Fungus."

The affected insects could generally be recognised by their colour, the green scale becoming white. They next turned greyish, and then darker and darker until they were almost black.

Under the microscope it was seen that in the white stage the body of the insect was filled with colourless spherical cells, which were present even in the legs and antennae. These were densely crowded, but towards the margin of the body they might be isolated and it could then be determined that they were not connected with any hyphae.

When the insect had become grey, it was found that the spherical cells in the middle of the body had germinated, giving rise to short stout hyphae (basidia), each of which bore at the apex a pyriform conidium, 18 × 9–10 μ. From Zimmermann's figures it would appear that the basidia were about 55 μ high, and 7–9 μ in diameter. The wall of the conidium was dark coloured, the dark colour of the diseased insect being due to the colour of the conidia. The conidia often germinated on the host; Zimmermann's figures show a conidium with three germ tubes.

Zimmermann stated that it seemed that the fungus might best be placed provisionally in the genus *Empusa*, although it was exceptional in that genus on account of the dark colour of the conidia and their germination by a germ tube.

The fungus was again recorded in 1918 by Coleman and Kunhi Kannan in "Some Scale Insect Pests of Coffee in South India" (*Dept. of Agriculture, Mysore State, Ento. Series, Bull. No.* 4). The early stages of its attack were shown by a whitish discoloration of the insect, but later it turned to a dark grey, while a greyish growth of fungus hyphae appeared on its surface. The conidia were oval. If the body of an affected insect was cut open, a mass of dark brown, spherical bodies was found inside. The fungus was not noticeable on coffee estates during the south-west monsoon, but occurred during the lighter north-east monsoon and during the early part of the drier cold weather. Under favourable circumstances, it might be found active until May, *i.e.* through the dry weather and almost up to the time of the heavy rains of the south-west monsoon.

Dr Coleman has kindly forwarded me specimens of the Indian species on *Lecanium viride* on guava, and I have similar specimens on *Lecanium viride* on coffee, Kotagiri, Nilgiris, *per* Mr R. D. Anstead, August, 1910. I have also similar specimens from Ceylon on *Lecanium viride* on *Futumia*, Peradeniya, October and November, 1913, and on a scale on *Cinnamomum ovalifolium*, Hakgala, February 27th, 1922.

In none of these specimens have I been able to find any *Empusa*. When the scale is in the white stage, it contains a very fine hyaline mycelium, 0·5–1 μ diameter, usually sparsely distributed. One or two hyphae may extend from the scale over the leaf and may bear a pyriform conidium at the tip. Numerous scattered spores of Dematiae, *e.g. Cladosporium*, etc., are usually present; and the scale may bear small patches, consisting of a few cells coalescent in one plane, which belong to the same fungi.

When the scale has become grey, it may be completely covered with the pyriform conidia. The fine mycelium is then abundant, but the hyphae remain separate, and do not fuse into masses. Hyphae and conidiophores of *Cladosporium* are usually present.

In general, however, when the scale darkens, the insect is permeated by the brown, irregular hyphae of one or more Dematiae. Moniliform chains of cells and small plate-like clusters are common, and conidiophores of *Cladosporium*, etc., arise at the margin. The fine hyaline mycelium with its pyriform conidia is present, but it is overgrown by the Dematiae.

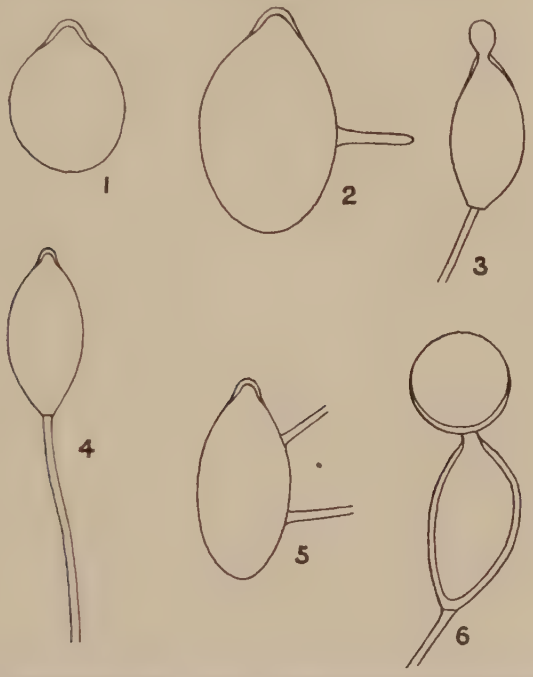
Finally, the whole scale is covered with a dense growth of conidiophores. In the Ceylon specimens these are for the most part *Cladosporium*, but in the specimen on guava from South India, a *Macrosporium* is the chief constituent. The scale at this stage is merely a thin film adherent to the leaf.

Thus, while the conidia of the hyaline mycelium become fuscous or pale brown, and might conceivably cause the scale to appear black, the black colour in all the available specimens is due to a dense growth of Dematiae on the dead scale.

The hyaline mycelium is very fine, 0·5–1 μ diameter, regular and not septate. The conidia are borne singly on free hyphae, or conidiophores, up to 60 μ long, 0·5–0·75 μ diameter, arising at the margin of the scale or anywhere on its surface. I have not seen a branched conidiophore. The conidia are narrow-oval or pyriform, with a small but well-marked papilla, and measure 12–20 × 5–10 μ (Fig. 4). A few may be broader and more globose, 12 × 9 μ (Fig. 1). They are thin-walled, except at the papilla. The scar of attachment is usually well-defined, and broader than the conidiophore. At first the conidia are hyaline, but they ultimately become fuscous or pale brown. These conidia are exactly those of a *Pythium* or a *Phytophthora*.

In general, the conidia germinate by the production of a germ tube, sometimes two, laterally (Figs. 2 and 5). The germ tubes are of the same diameter as the mycelium. In one collection, however, on *Lecanium viride* on *Futumia*, Peradeniya, November 5th, 1913, the papilla in very many cases has burst and the contents of the conidium (sporangium) have partly emerged

into a globose vesicle (Fig. 3). In one instance (Fig. 6) this vesicle forms a spherical spore-like body, 9 μ diameter, which appears to be persistent; the wall of this is thickened on the lower side. In some instances, there is a small swelling on the conidiophore a short distance below the conidium, but I have not observed any further development of this.



Figs. 1 and 4, sporangia; figs. 2 and 5, sporangia germinating; figs. 3 and 6, sporangia with vesicle. All × 1600.

The hyaline, globose spores noted by Zimmermann as occurring in the body and legs of the insect are not abundant in the available specimens. They have been observed in the specimen on *Futumia* noted above. They measure 8–10 μ in diameter, and are apparently attached to a very fine hyaline mycelium, similar to that which bears the oval or pyriform conidia.

In one collection, on a black *Aleyrodes* on *Cinnamomum ovalifolium*, the conidia are inequilateral. The mycelium is of the same dimensions as that described above.

Attempts to get this species into culture have failed, owing to the difficulty of separating it from bacteria and Dematiae. On the characters observed it would appear that it should be included in the genus *Pythium*. But it is aberrant in its fuscous

or pale brown spore wall, and in the formation of a persistent vesicle.

As I have not seen the type specimen of the fungus described by Zimmermann as *Empusa Lecanii*, it is not possible to make any definite statement concerning it. The recorded effect of *Empusa Lecanii* on its host is similar to that of the present fungus, and the conidia of the former, as described by Zimmermann, resemble those of the latter species in shape, size, and colour. Moreover, Zimmermann stated that the conidia of his fungus germinated with several germ-tubes, as in the present species. Consequently, it would appear that there is a strong probability that *Empusa Lecanii* is identical with the fungus described in this paper.

The correct position of this fungus can, however, only be decided by growing it in pure culture.

ENTOMOGENOUS FUNGI.

ADDITIONS AND CORRECTIONS, II.

(With 1 Text-fig.)

By T. Petch, B.A., B.Sc.

Moniliopsis rigida Petch, n.sp.

White to pale yellow, forming a loose, but somewhat rigid crust of interwoven hyphae, overlying colonies of mites. Hyphae regular, hyaline, 5 μ diameter, but varying in different lengths from 5 to 8 μ, bearing chains of spore-like cells terminally and laterally. Lateral chains simple, of 3–5 cells, arising either from a normal segment of the hypha, without any conidiophore, or from an intercalary polygonal cell which more or less resembles the cells of the chain; terminal chains of up to 6 cells, branching. Pseudoconidia or spore-like cells oval, truncate at both ends (except the terminal one of the chain), attached by a broad base, increasing in size towards the apex of the chain, 18–28 × 16–17 μ, not separating; wall stout, 1·5 μ thick.

On mites on *Phyllostachys bambusoides*, Brooksville, Florida, February, 1924, *per* J. A. Stevenson.

Rhinotrichum album Petch, n.sp.

White; conidiophores up to 0·1 mm. high, 1·5–2 μ diameter, rigid, erect, simple, or once dichotomously branched, at first in minute loose clusters, ultimately covering the insect with a loose white pile and spreading over the leaf; conidiophores hyaline,

rather closely septate, equal, slightly broader (2·5 μ) and clavate at the apex, bearing conidia everywhere on short sterigmata about 1 μ long; conidia globose or slightly oval, 1·5–2 μ diameter, hyaline, minutely verrucose.

On *Lecanium hemisphaericum*, Hakgala, March, 1922.

Cephalosporium falcatum Petch, n.sp.

Hyphae scanty, hyaline, 3 μ diameter: conidiophores in whorls of three, up to 26 μ high, inflated at the base to 2 μ diameter, strongly attenuated from half their height to the apex, heads oval, about 12 × 6 μ; conidia falcate, tips attenuated and acute, or narrow fusoid with the ends produced, straight or curved, continuous, 10–13 × 1·5–2 μ.

On a fly attached by the mycelium to the under side of a living leaf, Hakgala, March, 1922.

Coremium pulcherrimum Petch, n.sp.

Mycelium pinkish red, forming a network over the insect, sometimes uniting into strands. Synnemata arising at the margin of the scale or from the mycelium on its surface, up to 2 mm. high; stalk 0·4 mm. diameter, pinkish red, longitudinally fibrillose and rather loose, composed of hyphae 2 μ diameter, which separate above to form long conidiophores; head irregularly ovoid, loose, white, up to 1 mm. high, 0·9 mm. diameter; conidiophores long, 2 μ diameter, bearing dense whorls of phialides, about 20 μ apart on the uppermost 20 to 50 μ; phialides flask-shaped, 5 μ long, 2 μ diameter below; conidia catenulate, hyaline, oval, 2 × 1·5 μ, or globose, 2 μ diameter.

On *Lecanium nigrum*, etc., Peradeniya, August, 1921.

This species occurred on specimens of *Lecanium nigrum*, attacked by a parasitic insect, which were kept in glass tubes for the emergence of the parasite. The fungus attacked both the *Lecanium* and its parasite, developing on the latter after its emergence from the scale.

Coremium breve Petch, n.sp.

Synnemata up to 0·9 mm. high, with a stout, yellow, glabrous stalk, 0·4 mm. high, 0·25 mm. diameter, which expands into a white, loose, laterally compressed, circular head, 0·5 mm. diameter; some examples sessile, pulvinate, forming white powdery tufts, 1 mm. long, 0·6 mm. broad, at the margin of the scale. Conidiophores 2 μ diameter, branched, with lateral branches up to 30 μ long; heads of conidia terminal, the

conidiophores bearing short lateral branches just below the apex, which are crowned by flask-shaped phialides, 6 μ high, 2 μ diameter, forming a closely crowded head; conidia narrow-oval, hyaline, ends acute, 1·5–2 × 1 μ.

On *Lecanium* sp., Hakgala, March, 1922.

Coremium gracile Petch, n.sp.

White, clavate, shortly stalked, sometimes branched, up to 1·5 mm. high, 0·4 mm. diameter below, with an irregularly ovoid head, up to 0·7 mm. diameter; stalk loose, longitudinally fibrillose, composed of hyphae 3 μ diameter; conidiophores branched above, with two or three stout branches, 10 μ long, in a whorl; branches bearing whorls of stout subcylindric prophialides, 6 × 3 μ, slightly inflated at each end, which bear flask-shaped phialides, 7 × 2 μ; conidia hyaline, oval, one end acute, 1·5–2·5 × 1–1·5 μ, or subglobose, 1–1·5 μ diameter; heads of conidiophores up to 50 μ high, 40 μ diameter.

On a spider enclosed in a silk web, the coremia developing on the web, Peradeniya, July, 1921; Hakgala, March, 1922.

Fusarium entomophilum Petch, n.sp.

Mycelium white, sparse, overrunning the insect, and bearing purplish pulvinate acervuli, up to 1 mm. diameter: acervuli violet by transmitted light when fresh; conidia fusoid, ends falcate, or almost straight, four or five septate, 36–52 × 4–5 μ.

On *Clitellaria heminopla*, Suduganga, April 12th, 1919 (coll. R. Senior White).

Stilbum* (*Stilbella*) *coccorum Petch, n.sp.

Synnemata scattered, white, up to 0·3 mm. high, 65 μ diameter below, equal, or expanding above into a flattened-globose head up to 160 μ diameter; stalk minutely tomentose, sometimes forked above; conidia hyaline, globose, 1·5 μ diameter, or oval, 2 × 1·5 μ.

On *Fiorinia juniperi* on *Juniperus bermudiana*, Peradeniya, December 1923.

The synnemata arise at the margin of the scale, no mycelium being visible externally, but the scale is filled with white mycelium.

A species of *Stilbum*, *S. coccophilum*, was described by Saccardo (*Ann. Myc.* v, p. 178, 1907) on *Ceroplastes rusci* in Sicily. According to the description, the scale is covered with a mycelium from which the synnemata arise. The synnemata are up to 0·9 mm. high, filiform, with a glabrous stalk, 40 μ diameter, and a globose, glabrous head, 200 μ diameter. The head is not notably

attenuated into the stalk. The conidia are oblongo-fusoid, hyaline, somewhat acute, 6·5–7 × 2·5 μ.

I have not seen the type of *Stilbum coccophilum*, but it appears from the description to be quite distinct from the Ceylon species.

Hirsutella nodulosa Petch, n.sp.

Mycelium loose, grey; hyphae hyaline to pale brown, regular, septate, rather rigid, wall stout; basidia lateral, rarely terminal, simple, subcylindric, conoid, or flask-shaped, up to 16 μ high,

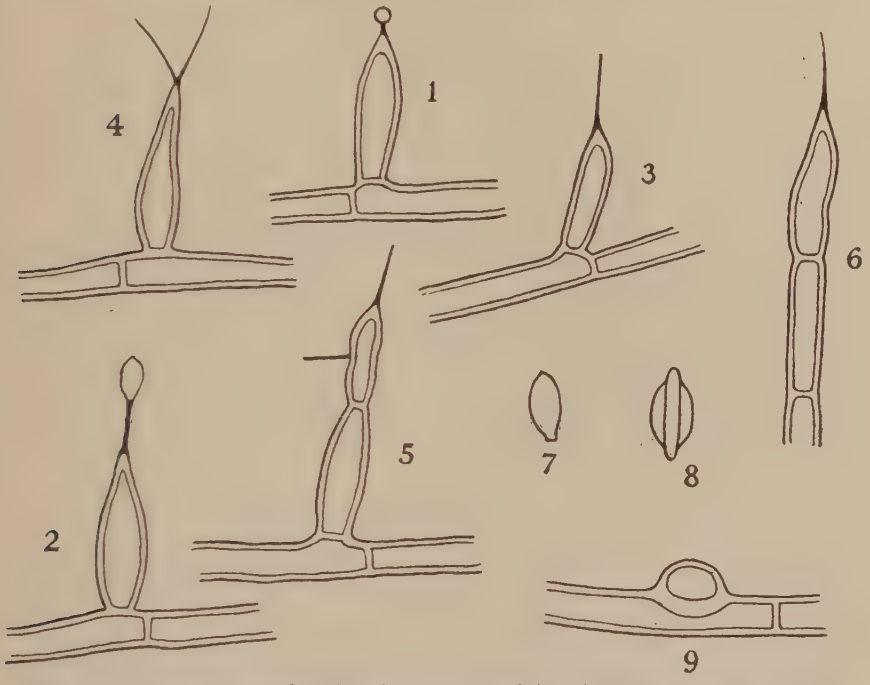


Fig. 1. *Hirsutella nodulosa* Petch. 1, 2, basidia with developing conidia; 3, basidium and sterigma; 4, 5, abnormal basidia; 6, a terminal basidium; 7, a single conidium; 8, a cluster of conidia; 9, swelling on a hypha. All × 1200.

3–4 μ diameter; sterigmata usually terminal and solitary, up to 16 μ high, 1 μ diameter, minutely nodular; spore clusters broadly oval, apiculate, 9 × 6 μ; individual conidia oval, apex subacute, base truncate apiculate, 6–7 × 3 μ.

On caterpillar of *Zeuzera coffeae*, Peradeniya, March, 1925.

Zeuzera coffeae is the Red Borer of coffee, tea, etc. In this instance, the caterpillar was found dead in its gallery in a twig of mahogany. From the nodular appearance of the longer

sterigmata, it would appear that the spore clusters are produced in succession on the one sterigma. Occasionally two sterigmata occur at the apex of a basidium, and sometimes one laterally in addition to the normal terminal sterigma. More rarely a basidium may be septate, or may give rise to another basidium laterally. Hemispherical protuberances occur commonly on the hyphae; these are subglobose or ovoid cells, the inner wall of which is strongly thickened and projects into the hypha.

Only one specimen of this species is available, and this differs from the type of *Hirsutella* in not possessing a clava.

Acremonium griseum Petch, n.sp.

Grey, or slightly lavender, forming a loose, pulverulent, floccose mass: hyphae hyaline, regular, thin-walled, 1–1·5 μ diameter; conidiophores lateral, simple, elongated conical or flask-shaped, up to 10 μ high, 1·5 μ diameter below; conidia terminal, hyaline, continuous, narrow-oval, 3–4 × 1–1·5 μ.

On spiders attacked by *Hirsutella* (?), Hakgala, March, 1922.

The insect is covered with grey or lavender floccose masses, and the mycelium spreads from it over the leaf, forming powdery patches covered with minute tufts. At first sight, the fungus appears to be parasitic on the insect, but further examination shows that one of the apparent legs of the insect is a decumbent clava, about 0·1 mm. diameter, which appears to be an abortive clava of a *Hirsutella*. The fungus is, as far as can be ascertained, parasitic on the *Hirsutella*, not on the insect.

Sporotrichum album Petch, n.sp.

Conidiophores suberect, arising from the repent mycelium in minute tufts, afterwards decumbent in white floccose masses, up to 50 μ long, simple or once branched, 1·2 μ diameter, somewhat geniculate above, widening at the angles to a breadth of 2 μ; conidia terminal or lateral, in the latter case sessile on the upper face of the angular projection, solitary, hyaline, continuous, cylindric, rounded above, subacute at the base, 6–10 × 1·2–1·5 μ.

Parasitic on *Cordyceps dipterigena* B. & Br. on *Mydaea* sp., Hakgala, March, 1922.

The fungus may be confined to the head of the *Cordyceps* or may cover the whole of the clava with a white floccose mass. It also spreads over the insect, and appears on the anterior margins of the wings; but as the insect is permeated by the hyphae of the *Cordyceps*, it is no doubt parasitic on the *Cordyceps* wherever it appears.

Tubercularia epimyces Petch, n.sp.

Sporodochia black, minute, pulvinate, gregarious or confluent, parenchymatous below, bearing closely-packed conidiophores; conidiophores with a simple base about 8 μ long, 2 μ diameter, branching above; branches about 6 μ long, bearing densely clustered, flask-shaped basidia, 8 μ long, at their apices; conidia hyaline, oval, 2 × 1 μ.

On *Aegerita Webberi* Fawcett on a scale insect on *Psychotria*, Hakgala, March, 1922.

The *Aegerita* is blackened, and from it fuscous hyphae radiate in a film over the leaf. These hyphae are 2 μ diameter, thin-walled, slightly irregular, and are united into strands. Here and there they bear blackish fuscous masses, about 50 μ diameter, which bristle with irregularly conical fascicles of hyphae, or single hyphae, up to 30 μ high. These masses may be developing pycnidia. The fungus has also been found on stromata of *Torrubiella*, Hakgala, September, 1923 (cf. p. 66).

Melanosphaeria circumdata Saw.

This new genus and species were described by Sawada in *Descriptive Catalogue of Formosan Fungi*, II, December, 1922. The description is in Japanese, but makes reference to *Aspidiotus fici* and *Microcera Fujikuroi*. From the figure, the species appears to be one of the black pycnidial fungi which are parasitic on entomogenous fungi, viz. either *Sirosphaera* (1913) or *Sirosperma* (1916).

Lycogala fragilis Holm.

This species, which occurred on a cockchafer (*Scarabaeus Melolontha*) was described by Holm in *Vid. Selsk. Skrifter*, Nye Samling, No. 1, p. 288, with fig. 5 (Kbh. 1781). Cooke, in *Vegetable Wasps and Plant Worms*, p. 108, wrote, "Nothing can be said of the so-called *Lycogala fragilis*, which was stated by Holm to have occurred on a broken and decayed specimen of *Melolontha vulgaris*, infesting the sides of the body and legs with small round spots. It was certainly not a *Lycogala*, but it may have been a *Laboulbenia*, or even *Leocarpus vernicosus*." As regards the insect, Holm merely stated that it was dead. From the description and figure there is little doubt that the fungus was a *Beauveria*.

Botrytis necans Massee.

This was described by Massee in *Kew Bulletin*, 1914, p. 159, from specimens on larvae of *Brachartona catoxantha* from Singapore. There is no type or other specimen in Herb. Kew.

Botrytis Rileyi Farlow.

This species was described by Farlow in the *Report of the (United States) Commissioner of Agriculture for the year* 1883, p. 121. It escaped inclusion in Saccardo, *Sylloge Fungorum*, and consequently has been unknown to mycologists in other countries, except through incidental mention, usually in entomological papers. During recent years it has been recorded on several occasions in the United States; and Dearness, in *Mycologia*, XVI (1924), p. 173, stated that it killed thousands of larvae of *Plathypena scabra* on beans in 1922. For convenience of reference, the original description may be quoted here.

"*Botrytis Rileyi* Farlow. Mycelium hyaline, diffusely branched, 1·5–2·5 μ in diameter. Spores in whorls, which are approximate at the ends of the hyphae and remote lower down. Whorls formed at the base of elliptical cells attached rather obliquely to the axis, developing into chains of, at first oval, and at length nearly spherical spores, 2–3 × 1·5–2 μ; when young whitish, becoming verdigris-green when mature. On larvae of *Plusia brassicae*, covering them with a distinctly green powder. This species is related to *Botrytis bassiana* Bals., which attacks silkworms, and from which the present species can easily be distinguished by the green-coloured spores. The chains of spores, although in general resembling those of *B. bassiana*, are more fully developed than in any form of that species."

This species would be exceptional in the *Beauveria* (*Botrytis*) *Bassiana* group in respect of its colour, and the description does not suggest *Beauveria*. If commas are inserted after the words "formed" and "base," the description agrees well with that of a *Spicaria*. The type specimen should be compared with *Spicaria prasina* (Maubl.) Saw. Watson (*Rept. Fla. Agric. Dept.*, 1915, p. xli) states that in Florida the caterpillar of *Anticarsia gemmatilis* on velvet bean is attacked by *Botrytis Rileyi*, the larvae turning first grey and then white. Specimens of a fungus on this caterpillar on the same host plant, sent to me from Florida, are green, and the fungus is *Spicaria prasina*.

Isaria stellata Cke.

This species was described by Cooke from specimens on mango leaves from Mysore, the description in *Grevillea*, IV (1876), 116, being "Nivea, stellata, incrustans. Floccis tenuissimis, circinatis. Encrusting dead insects attached to the under surface of mango leaves. Mysore. Snow white, encrusting minute insects and assuming the appearance of stellate bodies about 1 line in width. Threads very delicate, circinate, sigmoid or variedly curved. (Spores not seen.)"

In a *Report on Diseased Leaves of Coffee and Other Plants* (1876), Cooke wrote: "Specimens No. 4 of 'Mango leaves affected by a peculiar blight,' exhibited two forms of fungi, one black, occupying a large portion of both sides of the leaves, and the other of a snowy whiteness confined to the centre of the leaves on either side the midrib, on the under surface. The latter fungus in no way affects the tree, since it is not a parasite of the leaf itself, but was found investing minute dead insects attached to the leaf. These insects were so completely enveloped and metamorphosed by the snowy white woolly fungus (*Isaria stellata* Cooke, in *Grevillea*, IV) in which they were imbedded, that their precise character could not be ascertained, but probably they were some species of aphis, which is found to delight in Mango trees."

Cooke gave a further account of this species in his *Vegetable Wasps and Plant Worms*, pp. 306, 307. "Some Mango-leaves from India came into our hands some years since, and upon the under surface were found beautiful star-like, snow-white objects, almost like crystals of snow. Minute examination proved them to be minute insects, apparently *Aphides*, encrusted with an *Isaria*, about two millimetres in diameter, and sometimes confluent. The rays were numerous, radiating from a sort of discoid centre, regular, and not at all corresponding to any members, or projections, of the embedded insects; the latter impossible to extricate for identification. The whole mass of fungus was composed of delicate agglutinated threads. As often is the case, a considerable number of *Aphides* were congregated on the leaves, so that when they were close together, the encrusting *Isaria* united, becoming confluent in an irregular crust: but in cases where the insects were isolated, the star-like shape of the parasite was maintained."

The type in Herb. Kew is marked "on aphides." It consists of a group of insects which are furnished with waxy processes, such as commonly occur on leaves in the tropics, on which they form white patches. The type of *Isaria stellata* consists of the usual white, confused mass of insects and loose processes, with isolated insects which bear straight horizontal processes radiating from the margin of the insect over the leaf. The insect is *Phenacoccus mangiferae* Green. There is no *Isaria*. Apparently Cooke mistook the processes for a fungus.

Oospora Aphidis Cke & Massee.

This species was described by Cooke and Massee from specimens sent by Bailey from Australia. It was said to have catenulate, lemon-shaped, apiculate conidia, 17–19 × 12 μ.

In *Vegetable Wasps and Plant Worms*, p. 314, Cooke stated:

"Pumpkin-leaves, the under surface of which were attacked by Aphides, were sent to us from Queensland for examination, and we found that the dead insects were covered with a minute mould, having but very short and almost inconspicuous threads, but a profusion of spores, produced regularly in chains. In form they had resemblance to a lemon with an apiculus at each end, containing nuclei at first, but without colour, from seventeen to nineteen micro-millimetres in length, and twelve in breadth. The same mould did not appear on the leaves, apart from the insects, so that it is evident it did not spread from the plant to the dead insects (plate 2, fig. 21). At first there might seem a suspicion of this, since the species of *Oidium* and *Oospora* are common, and injurious to the foliage of living plants."

Bailey sent the specimen because it bore an *Oidium*. The mycelium of the *Oidium* covers the leaf, and bears typical conidia, 28–40 × 15–18 μ, sometimes in chains. The aphides bear scattered conidia, either globose, 15–16 μ diameter, or oval, 16–18 × 12–15 μ, broadly apiculate; these are the conidia of an *Entomophthora*. Cooke and Massee's measurement was probably based on the conidia of the *Entomophthora*, but the figure in Cooke's *Vegetable Wasps and Plant Worms*, which shows catenulate conidia, was more probably derived from the *Oidium*. The name *Oospora Aphidis* is to be discarded.

ON A NEW SPECIES OF UROPHLYCTIS PRODUCING GALLS ON LOTUS CORNICULATUS LINN.

(With 26 figs. arranged as Plates XI—XIV.)

By A. W. Bartlett, M.A., M.Sc.

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In the summer of 1921 my attention was directed by Professor M. C. Potter to some peculiar gall-like structures of fungus origin, which he had observed about eighteen years previously on the bird's-foot trefoil (*Lotus corniculatus* Linn.). A search for these in the field where they were originally discovered proved fruitless, but they were found in another field, distant about half a mile. Even here the galls were by no means plentiful, and to obtain specimens of them a careful and thorough search was required. About the same year in which he first found the galls, Professor Potter discovered another locality for them in a field separated from the previous station by a distance of about

thirty miles. We visited this spot together recently, but were unsuccessful in procuring any specimens. A careful and diligent search in other fields where *Lotus corniculatus* grows has not so far yielded any examples.

Thus, at the present time, only one locality is known for these galls, and this is situated at about eleven miles nearly due north-west of Newcastle-upon-Tyne. The infected plants of bird's-foot trefoil grow in the lowest and dampest part of a pasture field.

Description of the Galls.

The galls occur usually in the region of the "collar," so that they are situated just above the surface of the soil, but occasionally they are found upon the creeping underground stems. A shoot seldom bears more than one or two, but sometimes three, or even four, of them are found together, and they are usually situated laterally upon the stems. They have a more or less spherical or ovoidal form, and are rarely lobed. The situation and appearance of the galls is shown in Fig. 1. The largest galls measure about 1 cm. in the longest diameter, but the average size is that of a fair-sized garden pea. On the underground stems some very small ones were found, about 1 mm. in diameter, and bearing some resemblance to root-nodules.

The colour of the galls, when freshly obtained, is a pale brown; the surface is smooth and slightly scurfy. The very young ones are of a pale yellow colour.

The gall-bearing plants appear to be as healthy and to reach the same size as ordinary individuals. They produce no flowers, but this is usually the case with plants of *Lotus corniculatus* when growing in rather moist situations. Plants of *Lotus uliginosus* Schk. which grew in the same spot, were entirely free from galls.

The cut surface of a gall has a white and brown mottled appearance. A section cut by hand and examined under the microscope discloses a parenchymatous tissue consisting for the most part of thin-walled cells, and numerous rounded cavities of various shapes and sizes mostly filled with brown resting sporangia (Figs. 2–4). The hemispherical form of the latter, the scarcity or almost entire absence of hyphae, as well as the general structure of the gall, are all characteristic of the Chytridiaceous genus, *Urophlyctis*, of which this is apparently a new species.

Methods.

Small pieces of the galls were fixed in Carnoy's fixing fluid, in corrosive sublimate acetic acid alcohol, in medium chrom-acetic solution, and in Flemming. The microtome sections were stained with diamant fuchsin and light green, or with Haiden-

hain's iron-alum method, using orange G in clove oil as a contrast stain, or with Flemming's triple stain. The best results were obtained with the first two fixing media followed by Haidenhain's iron-alum method. Hand-cut sections stained with cyanin and orange G in clove oil were also very useful, as well as preparations made by teasing up the fresh galls in water on a slide.

Microscopic Structure of the Gall.

The gall is composed principally of thin-walled parenchymatous cells containing a nucleus and abundant starch grains; irregular vascular bundles, in which short tracheids with transverse pits are conspicuous, traverse the gall in various directions. There is no definite epidermis or any signs of the formation of a periderm, but the outermost cells are mostly empty and dead.

The cavities containing the resting sporangia (Figs. 2 and 3) occur sometimes apparently isolated, sometimes in groups, the separate chambers being mostly in open communication with one another, or separated only by intervening cell walls. I think it probable, however, that the apparently isolated chambers are linked up with others situated either above or below, so that the system of cavities forms one connected whole.

The cavities show a considerable diversity in form and size, but their contour is usually more or less rounded. The walls by which they are bounded are thicker and more distinct than the ordinary cell walls (Figs. 16 and 17). The cavities themselves appear to be formed by the breaking down and digestion of the walls of cells, the remains of the protoplasm and nuclei of which (Figs. 16 and 17, *n*.) are often to be seen between or around the immature resting sporangia.

The Resting Sporangia.

A mature resting sporangium has the form of a hemisphere with a slightly convex base (Figs. 5 and 20), but after drying or immersion in spirit the base becomes concave, thus agreeing with the descriptions and figures of other species of *Urophlyctis*. Attached to the centre of the base of the sporangium by a very short stalk is a small transparent vesicle or cell (not shown in Figs. 5 or 20), with a very fine hypha springing from the pole opposite to the sporangium. A resting sporangium has a diameter of 40–50 μ and a height of 25–30 μ. The wall is of uniform thickness (4 μ) except at the angle formed by the meeting of the two curved surfaces where its thickness is increased; it is of a pale yellowish brown colour. In it two layers can be distinguished, a thick, coloured exospore, and a thin, colourless endospore (Fig. 21). The exospore is hard and brittle, almost glassy

in texture, so that in microtome sections it is frequently found broken into irregularly shaped pieces.

The more convex surface of the resting sporangium is ornamented with an irregular pattern of conspicuous grooves (Figs. 5, 6 and 14), varying in form, length and width, but their principal direction is from the apex downwards. The base is finely granulate, with a small pit in the centre, marking the spot where the colourless vesicle is attached.

The immature resting sporangia, which may be recognised by their paler colour and less conspicuous markings, have the apex smooth, and when boiled in caustic potash and afterwards cleared in chloral hydrate, show when examined under a one-twelfth inch oil immersion objective a rather irregular ring of very fine pores surrounding the smooth apex (Fig. 6). The number of these varies from five to nine, and is most usually six. It is almost impossible to distinguish these pores on the mature resting sporangium, on account of the surface ornamentation which covers the apex. Through each pore passes a small, branched, haustorium-like process (Figs. 7 and 10–13) while the sporangium is growing, but these processes disappear when the sporangium has reached maturity.

From the examination of the resting sporangium of a number of species of both *Urophlyctis* and the closely related genus, *Physoderma*, Jones and Drechsler (9) concluded that a certain range in the number of these pores is characteristic for each species. For example, six or eight were found in *Urophlyctis Rubsaameni*, nine to fifteen in *U. Alfalfae*, and fourteen to twenty-four in *U. pluriannulatus*.

On account of conflicting statements made by Saccardo and Mattirolo (22) and by Lagerheim (10) respectively with regard to the composition of the wall of the resting sporangium of *Urophlyctis Alfalfae*, I tried the usual micro-chemical tests for cellulose and cuticularised walls on the resting sporangia of the present species. In every case I obtained negative results for cellulose or cutin. Probably the wall consists principally of chitin, which appears to be the case in most of the Archimycetes.

Lagerheim (*loc. cit.*) also reported that the walls of the cavities containing the resting sporangia of *U. Alfalfae* were lignified, whereas Saccardo and Mattirolo (*loc. cit.*) found that they consisted of typical cellulose. The latter is certainly the case with the species under discussion. Scattered about, however, in the sections of the galls were isolated cells or small groups of cells, destitute of contents, which had probably previously contained the parasite, and the walls of these cells gave characteristic lignin reactions.

Incidentally, it may be mentioned that, while using cyanin

as a test for cuticularisation, I found that this stain proved very useful for showing up immature stages of the parasite in hand-cut sections of the galls. These became a deep blue colour, but the nearly or quite mature resting sporangia remained unstained.

Development of the Parasite.

The development of *Urophlyctis* has been investigated by Schroeter (24), Magnus (11–14), Maire and Tison (18), Vuillemin (31) and others, and most recently by Jones and Drechsler (9), who have furnished some beautiful figures of large portions of the thallus, skilfully dissected out of the fresh galls. The course of development is as follows. As the result of infection of an epidermal cell of the host by a zoospore of the parasite, the protoplasm of the zoospore is seen as a small rounded body within the cell attacked. The process of infection has not been actually observed by anyone, but probably takes place in exactly the same way as in certain other species of Chytridiineae in which it has been studied. In these the zoospore, after a period of swarming, becomes attached to its host, surrounds itself with a membrane, and then puts out a very fine infection-tube by means of which it pierces the external wall of its host. Through this tube the contents of the zoospore gradually pass into the epidermal cell of the host. After infection is completed, the empty wall of the zoospore is often visible on the external surface of the host.

This minute fungus cell increases in size, and develops a branched haustorial process at its apex. This cell gives rise to a small number of very fine hyphae with slightly swollen ends. With the growth in length of these hyphae, the swollen apices also increase in size, until, finally, each one comes to resemble the parent cell and is likewise terminated by an haustorium. This proceeding is repeated a few times. Each cell also normally gives rise to a resting sporangium, which arises as a small round body from the centre of the haustorium, and rapidly increases in size. It draws its nourishment from two sources, partly from the cell from which it originates, and partly directly from the host by means of a ring of haustoria, situated near the apex, to which reference has already been made.

To the cells from which the fresh hyphae and the resting sporangia arise various names have been given. Büsgen (3) termed them "Sammelzellen," A. Fischer (6) speaks of them as "Anhangszellen," Cornu employs the name "corps central," Vuillemin (31) speaks of them as "vesicules collectives," Magnus (14) designates them "antheridia," while Jones and Drechsler (9) describe them as "turbinate cells." In this paper I prefer to

make use of Büsgen's original term "Sammelzellen," which I translate as "collecting cells."

My own observations, made upon preparations obtained by teasing apart small pieces of the tissue of the fresh galls at different stages of growth, show that the general structure and development of the present fungus agree closely with other species of *Urophlyctis*, except in a few minor details. Sections, either stained or unstained, are unsatisfactory for this purpose, because the various stages in the development of the parasite are so closely crowded together within the cavities of the galls that it is impossible to observe how they are connected.

The mode of infection could not be observed, because by the time a gall has developed sufficiently to become visible infection is long past, and the few external layers of cells of the galls were always quite free from the parasites. But the presence of empty cells here and there, the walls of which reacted to stains differently from those of the other cells, made it probable that they had previously harboured stages of the parasite. The hyphae are extremely narrow, measuring about 5 μ in diameter, and, as is characteristic of the genus *Urophlyctis*, these hyphae either disappear after their function is finished, or at least they can no longer be discerned. An exception to this last statement is furnished by the occurrence in the older galls of several hyphae with greatly thickened and irregular walls. These were also observed by Magnus (14), Vuillemin (31), and Jones and Drechsler (9). Magnus suggested that these thick-walled hyphae may renew their growth during the next period of vegetation. They appear, however, to be rather undergoing degeneration, and, in any case, one does not see the necessity for this mode of perennation when there is such an abundant production of resting sporangia, presumably capable of germination under suitable conditions.

The collecting cells (Figs. 7 and 10–13) have a somewhat top-shaped form, being rounded at the distal end which bears the haustorium, and tapering at the proximal end where they are attached to the hypha. They measure 15–20 μ in diameter. They are at first filled with dense granular contents. When the resting sporangium arises from the haustorial process, as already described, branches of the haustorium are often visible around the base of the sporangium during the early stages of its growth (Fig. 13). The contents of the collecting cell are transferred into the growing sporangium leaving the collecting cell empty, except for a few scattered granules. The empty collecting cell is visible for a long time as a small round transparent vesicle attached to the less convex side of the resting sporangium.

Schroeter (24) was the first to observe the phenomenon described above, which he interpreted as a sexual act, and he termed

the collecting cell the antheridium, and the young resting sporangium an oosporangium. But there is insufficient evidence to support Schroeter's views.

The young resting sporangium is colourless and its external surface is smooth. It has the form of a flattened sphaeroid, and its apex is covered with the haustorial processes to which reference has previously been made (Figs. 7, 10–13). Its contents appear coarsely granular with abundant oil drops. As it increases in size the basal part becomes flatter (Figs. 9, 10), the markings upon its surface make their appearance, and the circular ridge separating the two surfaces develops. Later on, the wall increases in thickness and becomes brown, and the ornamentation of the surface is more pronounced.

A collecting cell gives rise also to fresh collecting cells, which spring from near the apex as a small number of fine hyphae with enlarged ends each terminating in an haustorial process (Figs. 9–11). In the present species the maximum number which arises from any given collecting cell is three, but in *Urophlyctis Alfalfae* and *U. pluriannulatus* the maximum number, according to Jones and Drechsler (9), is respectively four to five and seven to eight.

Often, only one or two were observed, but others may have become detached during manipulation. A collecting cell usually gives rise to others before the resting sporangium which it bears has nearly reached its full size.

In their account of the development of *Urophlyctis Alfalfae* and *U. pluriannulatus*, Jones and Drechsler (9) state that, before the collecting cell gives rise to others of a higher order, a corresponding number of peripheral lens-shaped cells are cut off from it in the neighbourhood of the apex by means of curved septa, and a fresh collecting cell arises from each of these segments. After long and careful observation, I have been unable to verify this proceeding in the present species. But I have observed several times in the teased preparations and in microtome sections that a fair-sized segment is cut off by a wall on one side of the collecting cell (Figs. 7 and 13). In one or two instances I found two segments delimited in this way on opposite sides from the upper part of a collecting cell (Figs. 12 and 19). When a segment (or two segments) was cut off in this manner, in no case did the segment or segments appear to give rise to fresh collecting cells and I am unable to explain their significance. The hyphae very rarely branch, but one instance of this was seen (Fig. 13).

Origin of the Cavities within the Galls.

While the parasite is growing and producing new sporangia, it is continually enlarging the cavities in the galls within which it is contained. The cell walls are the first to be broken down and dissolved, and the remains of the protoplasm and nuclei of the host cells may be seen around and among the immature collecting cells and resting sporangia of the parasite (Figs. 16 and 17). By the time all the sporangia in any cavity have reached maturity no traces remain of the host protoplasm or nuclei, since these have been used up by the parasite.

The effect of the fungus upon the nuclei of the broken-down cells is to cause a considerable enlargement of the nucleus as a whole, and of the nucleolus, which renders them very conspicuous (Figs. 16 and 17, *n.*). Bally (1) noticed the same phenomenon in *Urophlyctis Rubsaameni*, and Jones and Drechsler (9) in *U. Alfalfae*. Von Guttenberg (8) observed a similar hypertrophy of the nucleus of host cells inhabited by species of *Synchytrium*.

The wall lining the cavity is considerably thickened, but it still gives a cellulose reaction. The walls separating neighbouring cavities often show window-like openings of various shapes and sizes where the wall is in process of being broken down (Figs. 24 and 25). Magnus (12) describes and figures similar perforations in the case of *Urophlyctis pulposa*. All the cell walls of the galls stain deeply with ruthenium red, proving the abundant presence of pectic compounds.

The conclusion that I have arrived at from the study of galls at various stages of development is that their growth is brought about mainly by the activity of the cambium of the stem, aided by the cambium of the numerous vascular bundles which traverse the galls. There is never any cambial activity producing radial rows of numerous cells surrounding the cavities, such as Magnus (12, 14) describes and shows in his figures of *Urophlyctis leproides* and *U. Alfalfae*.

Temporary Sporangia.

No temporary sporangia have been observed in the present species. The only member of the genus for which they have been described is *Urophlyctis pulposa*, by Schroeter (24), but Magnus (12) and Vuillemin (31) failed to find them. They were said by Schroeter to be of a huge size (up to 200 μ across), spherical or ovate in form, with bright orange contents. Beneath each sporangium is a peculiar enlarged cell into which penetrates a dense tuft of short branched hyphae constituting an haustorium (*vide* also Schroeter (25), Fig. 70). The sporangium is surrounded

by an investment formed by the growth of the surrounding epidermal cells, suggesting, apart from the haustorium, the gall formed by certain species of the genus *Synchytrium*. The zoospores which escape by a pore at the apex, are described as spherical in form and comparatively large, with one cilium and a refringent oil-globule. This description fits exactly the zoospores also of *Synchytrium*.

Germination of Resting Sporangium.

Attempts made to germinate the resting sporangia so far have not been successful. They have been sown in water and in various culture solutions, kept at various temperatures. They have been subjected to artificial freezing. The galls were observed to have been occasionally gnawed by slugs, and it was thought that possibly slugs might be instrumental not only in the dissemination, but also in bringing about the germination of the sporangia. Elliott (4) has made some observations on the mycophagous propensities of slugs. A few specimens of the common field slug (*Agriolimax agrestis*) were first subjected to a short period of starvation to empty the alimentary canal, after which they readily devoured the galls. The faeces consisted almost entirely of resting sporangia, but attempts made as before to germinate these gave negative results. Bally (1) states that he has observed the germination of the resting sporangia of *Urophlyctis Rubsaameni*, which took place readily after they had lain for some time in water. An active movement of the zoospores was first visible in the interior of the sporangium. Irregular clefts then appeared in the exospore, followed by the rupture of the exospore first, and then the endospore. The escaping zoospores had a diameter of 1–3 μ and were provided with a single cilium. They performed rotating movements similar to those of *Synchytrium endobioticum*, and exhibited in their cytoplasm two points coloured black by the vapour of osmic acid. The resting sporangium never became completely emptied, but a certain amount of protoplasm with oil drops remained behind.

In the autumn of 1922, plants of bird's-foot trefoil bearing galls were planted in a flower-pot, and left outside during the winter to ascertain whether, after a lengthy exposure to atmospheric influences, the resting sporangia could be induced to germinate. The flower-pot, however, unfortunately met with an accident. A similar experiment was again tried during the winter of 1924–5, but no germination could be obtained when the resting sporangia were sown in water and nutrient solutions during the following spring and summer.

Cytology.

In the youngest stage found of the collecting cell a single nucleus only was present, which it probably received from a collecting cell of a lower order, according to the observations of Jones and Drechsler (9). As the collecting cell grows in size the nuclei within it increase in number. Figs. 8, 26, and Figs. 16 and 17, *c.c.* represent collecting cells in different stages of development. Within each nucleus of the collecting cell is seen a deeply staining, round or oblong nucleolus or karyosome, usually occupying a more or less central position. Surrounding this is a clear area, the nuclear vacuole, which appears to be delimited externally by a nuclear membrane. The fine hyphae which join together the collecting cells contain no nuclei. With reference to the mode of multiplication of the nuclei, occasionally figures were seen which suggest an indirect division with the formation of a spindle (Fig. 15), but on account of the very small size of the nuclei in the collecting cells nothing definite can be stated. Bally (1), Fron and Lasnier (7), Maire and Tison (18), and Jones and Drechsler (9), all agree in describing the nuclear divisions as amitotic. The surrounding cytoplasm is dense and finely granular, and stains somewhat deeply with nuclear stains. It is bounded externally by a very delicate cell wall.

When the resting sporangium arises as an outgrowth from the centre of the haustorium, at the apex of the collecting cell, the nuclei and cytoplasm contained in the latter are gradually transferred into the young resting sporangium. Fig. 22 represents a stage in the process, in which a nucleus is seen squeezing its way through the narrow connection between the resting sporangium (*r.s.*) and the collecting cell (*c.c.*). Finally, the collecting cell becomes entirely emptied of its contents.

Very soon after the nuclei have passed into the young developing resting sporangium, a number of them undergo a considerable increase in size, which involves not only the nucleus as a whole but also the nucleolus. The nucleolus is then found in contact with the nuclear membrane, and a distinct chromatin network is seen occupying the nuclear vacuole. The nuclear membrane becomes thickened and very clearly defined (Figs. 16, *r.s.*, 18, 19, and 21). Either the enlargement of the nuclei takes place very rapidly, or the process of transference of the contents of the collecting cell is a slow one, because in Fig. 22 one of the nuclei in the resting sporangium is seen to have become considerably enlarged whilst the collecting cell has lost only a part of its contents. These nuclei attain a diameter of 5 to 6 μ and their nucleoli measure 2 to 2·5 μ across. Concurrently with the enlargement of the nuclei, spherical grains of chromatin of various

sizes, staining a deep black with iron haematoxylin, make their appearance scattered throughout the cytoplasm of the developing resting sporangium (Figs. 16, *r.s.*, 18, 19 and 21). The appearance of granules of chromatin in the general cytoplasm, as well as the enlargement of some of the nuclei and nucleoli, are likewise reported as occurring in *Urophlyctis hemisphaerica* and *U. Alfalfae* by Maire and Tison (18) and Jones and Drechsler (9) respectively. These observers state that the origin of the chromatin in both of the above species is due to the swelling and final disintegration of some of the nuclei. I have been unable to confirm this observation in the case of the present species, but am of opinion that the nuclei diminish in number while the resting sporangium is increasing in size. At the same time the general cytoplasm becomes less dense and shows a distinctly reticulate appearance. In the mature sporangium the granules of chromatin collect in the centre to form a large deeply staining mass of irregular form (Figs. 17, *r.s.*, 20 and 23). The nuclei are found situated outside this mass, and are often difficult to observe.

In the meanwhile, the resting sporangium loses its almost spherical form, and becomes transversely ellipsoidal and its wall becomes thickened and acquires the characteristic ornamentation; afterwards, the future base undergoes a still further flattening. The wall does not increase uniformly in thickness throughout, but at first the thickening is most pronounced at the base and apex (Fig. 21), the circumference remaining thin for a time and thus allowing for further extension.

In the mature sporangium (Fig. 20) the wall is thickest in the centre of the base and apex, and somewhat thinner around the circumference immediately above the prominent ridge where it acquires its greatest thickness. Two distinct layers are visible in a section through the wall, the endospore and the exospore (Fig. 20, *en.* and *ex.*).

General Considerations, and Systematic Position of the Parasite.

The fungus evidently belongs to the family Cladochytriaceae A. Fischer (*sensu restricto*), which in a critical survey of the entire group of the Chytridiineae, not yet published, I have characterised as follows:

"Mycelium well-developed, consisting of simple or branched hyphae, which bear several either terminal or intercalary sporangia. Sporangia of two kinds, temporary, or resting, both formed asexually. Hyphae usually disappearing when the sporangia are mature. Mostly parasitic within the tissues of seed-plants."

Four genera are included in this family, viz. *Cladochytrium*, *Physoderma*, *Urophlyctis*, and *Nowakowskiella*. The characters by which these genera are distinguished from one another have not been very clearly defined in the past with the result that certain species have been shifted about from one genus to another by different authorities. The life history, also, of most of the species is very imperfectly known.

A. Fischer (6) placed all the species then known to him in the single genus *Cladochytrium* which comprised the three subgenera *Cladosporangium*, *Urophlyctis*, and *Physoderma*. There is much to be said for his point of view, because all the members of this family appear to be closely related. However, he based the diagnosis of his three subgenera principally upon the presence or absence, and the characters of the zoosporangium or temporary sporangium. Since the latter has been reported for only a single species of *Urophlyctis*, and the isolated observation has not been confirmed, it does not seem to me to afford satisfactory or safe characters to be used for the purposes of classification.

Schroeter (25), on the other hand, claimed the existence of four separate genera, but he placed the genus *Urophlyctis*, founded by himself, in a separate family, Oochytriaceae, on account of a supposed sexual process in the formation of the resting sporangium, to which reference has already been made. Even if there were sufficient evidence to support Schroeter's assumption, little can be said in defence of this arrangement, because it removes *Urophlyctis* from the other genera, to which it is evidently closely related, and places it in a heterogeneous group containing the genera *Diplophysa* (= *Olpidiopsis*), *Polyphagus*, and *Zygochytrium*, with none of which does it exhibit near resemblances in its other characters. Thus, the above is a purely artificial classification.

Magnus (13) shared Schroeter's views on the occurrence of a sexual process in *Urophlyctis*. He published detailed accounts of several new species of *Urophlyctis* (11–15) and did much to increase our knowledge of the genus. As the result of his investigations, he came to the conclusion that the hemispherical form of the resting sporangium, to the lower more or less flattened surface of which is attached a colourless empty cell, furnished a character by which the genus *Urophlyctis* might be distinguished from the closely allied genus *Physoderma*. In the latter the resting sporangium is uniformly oval, and the collecting cell is attached at any point. Furthermore, he pointed out that *Urophlyctis* causes a considerable enlargement of the host cells, the walls of which become swollen and mucilaginous, and are eventually partially absorbed. But this character does

not apply to all the species, because in *Urophlyctis Urgineae* (Pat. & Trab.) Maire, if indeed this is a *Urophlyctis*, the walls of the host cells appear to be not at all affected.

The present fungus, then, should be included in the genus *Urophlyctis*, as defined by Magnus, which contains the following species:

1. *U. major* Schroet. Krypt. Fl. Schles. III, i, Pilze, p. 197, 1886. In leaves of *Rumex* sp.
2. *U. pulposa* Schroet. Ibid. In leaves, stems and perigonia of *Chenopodium* spp. and *Atriplex* spp.
3. *U. Alfalfae* (Lagerheim) Magn. in Ber. Deutsch. Bot. Ges. XX (1902), 291–296. On *Medicago* spp.
4. *U. Rubsaameni* Mag. Ibid. XIX (1901), (145)–(153). In roots of *Rumex scutatus*.
5. *U. Trifolii* (Pass.) Magn. in Centralbl. f. Bakt. IX (2) (1902), 895–897. On leaves, petioles and peduncles of *Trifolium* spp.
6. *U. hemisphaerica* (Speg.) Syd. in Ann. Mycol. I (1903), 517–518. On leaves of several species of Umbelliferae.
7. *U. punctiformis* Spegazzini in Ann. Mus. Nac. Buenos Aires, XVIII (1909), 284. In leaves of *Hypochaeris variabilis* and *Picrosia longifolia*.
8. *U. Magnusiana* Neger in Ann. Mycol. IV (1906), 280–282. In stems, leaves and calyces of *Euphrasia Odontites*.
9. *U. Urgineae* (Pat. & Trab.) Maire. Bull. Soc. Bot. France, LIII (1906) clxxxiv. In leaves of *Urginea Scilla* and *U. maritima*.
10. *U. pluriannulatus* (Berk. and Curt.) Farlow in Rhodora, X (1908), 12–13. In leaves, petioles and stems of *Sanicula* sp. and *Ligusticum apiifolium*.
11. *U. Asphodeli* (Debray) Maire in Bull. Soc. Bot. France, LIII (1906), clxxxiii. In leaves of *Asphodelus microcarpus* and other species.

If we examine the hosts in or upon which the above species occur, we shall observe that the genus *Urophlyctis* exhibits a somewhat closely specialised parasitism, since each species is confined to either a single genus of flowering plants, or, at the most, to a few genera belonging to the same Natural Order. Furthermore, each species attacks a definite part or parts of the host. Hence, it is unlikely that the present species, parasitic upon *Lotus corniculatus*, would attack other than Leguminous plants.

Of the species of *Urophlyctis* already described two only are found upon plants belonging to the Leguminosae, viz. *U. Alfalfae* on Lucerne and another species of the genus *Medicago*, and *U. Trifolii* on certain other species of *Trifolium*. *U. Trifolii* may be dismissed from our consideration, because it attacks only the subaerial parts of the host, *i.e.* the leaves, petioles and peduncles, resulting in the production of minute hemispherical warts. Moreover, the wall of the resting sporangium is described as smooth, as it is also in *U. Alfalfae*.

Further characters in which *U. Alfalfae* differs from the species on *Lotus corniculatus*, are found in the large irregular coral-like galls produced by the former, and the much smaller, smooth, rounded galls caused by the latter. Differences in the structure of these two kinds of galls have already been pointed out. Other differences may be noted in the resting sporangia.

The number of pits in the wall through which the sporangia emerge were found by Jones and Drechsler (9) to be nine to fifteen for *U. Alfalfae*, and they are described as forming a circle which occupies a position a little above the equator. The pits number five to nine in my species, and they form an irregular circle situated much nearer to the apex.

Lastly, the present species differs from all those previously described in the characteristic ornamentation of the surface of the resting sporangium, as well as in the distinct angular ridge separating the more convex from the flatter surface. In the accounts or figures of all the other species of *Urophlyctis*, the external surface is described or represented as smooth, the wall as of even thickness throughout, and the zone between the upper and under surfaces as rounded.

From the above considerations, I believe that I am justified in regarding the *Urophlyctis* producing galls on *Lotus corniculatus* as a new species, to which I propose to give the name of *Urophlyctis Potteri*, after Dr M. C. Potter, Professor of Botany at Armstrong College, who first drew my attention to these galls. It may also be regarded as a slight token of my affection and esteem for Professor Potter, who has recently retired after thirty-five years of arduous and distinguished service as Professor of Botany at Armstrong College.

Urophlyctis Potteri sp.nov.

In basi caulium erectorum aut in caulibus hypogaeis Loti corniculati generans excrescentias globulosas usque ad 1 cm. diam., excrescentias plenas sporangiis perdurantibus in variis lacunis, sporangiis hemisphaericis sub-brunneis exornatis supra inordinatis radiantibus sulcis, infra convexiusculis granulatisque, 40–50 μ diam. 25–30 μ altis.

Hab. In basi caulium Loti corniculati prope Ponteland, Northumberland, England.

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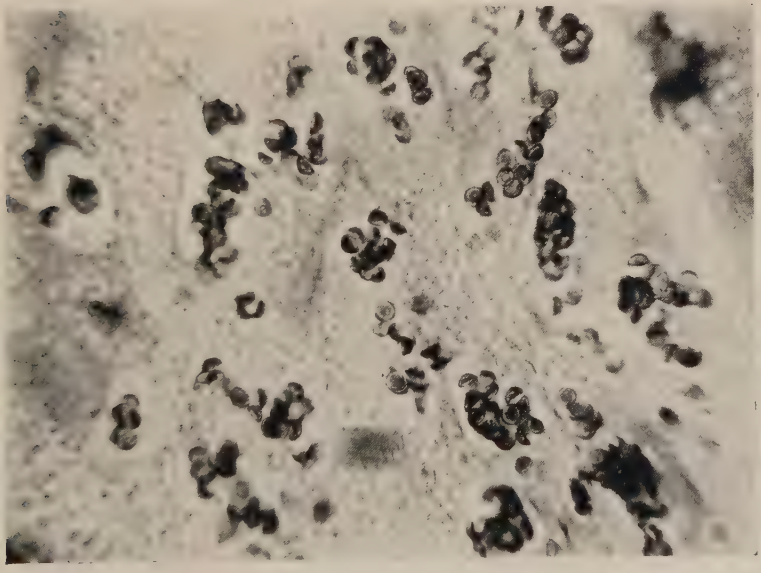
DESCRIPTION OF PLATES XI—XIV.

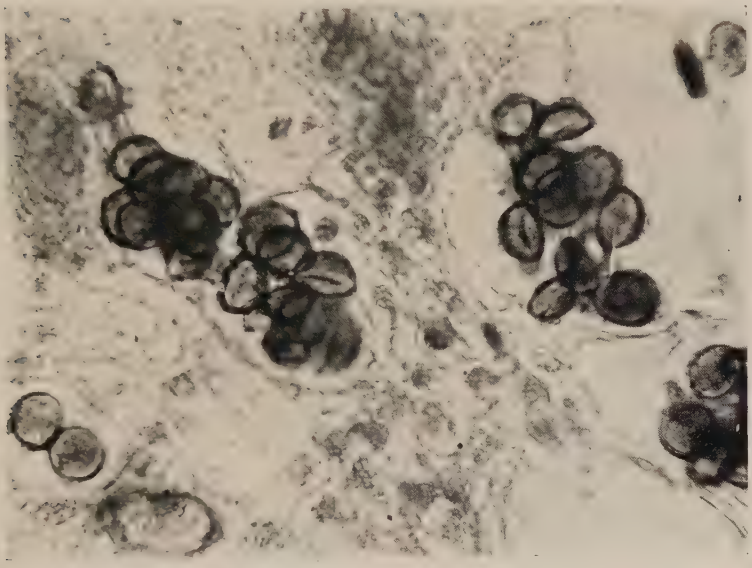
Fig. 1. Photograph of galls on plants of *Lotus corniculatus*. Nat. size.
Fig. 2. Photomicrograph of section of gall. × 50.
Fig. 3. Photomicrograph of section of gall. × 150.
Fig. 4. Photomicrograph of a cavity in a gall containing resting sporangia. × 350.

1



2

UROPHLYCTIS POTTERI



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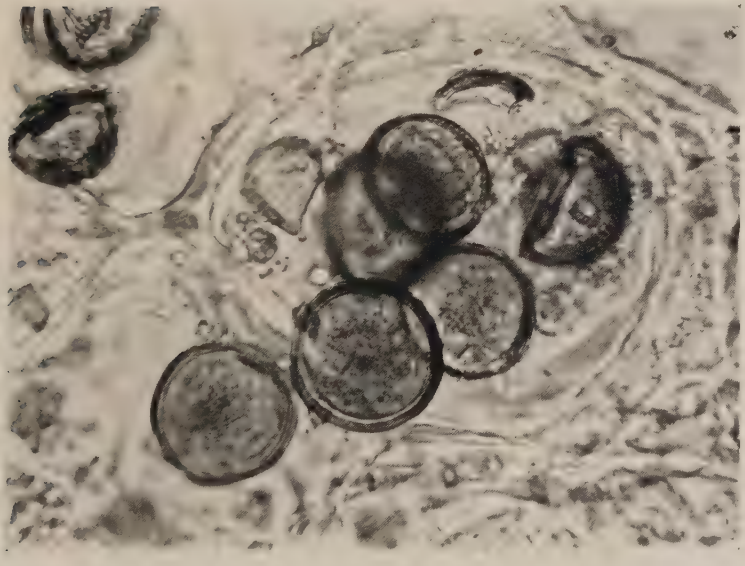

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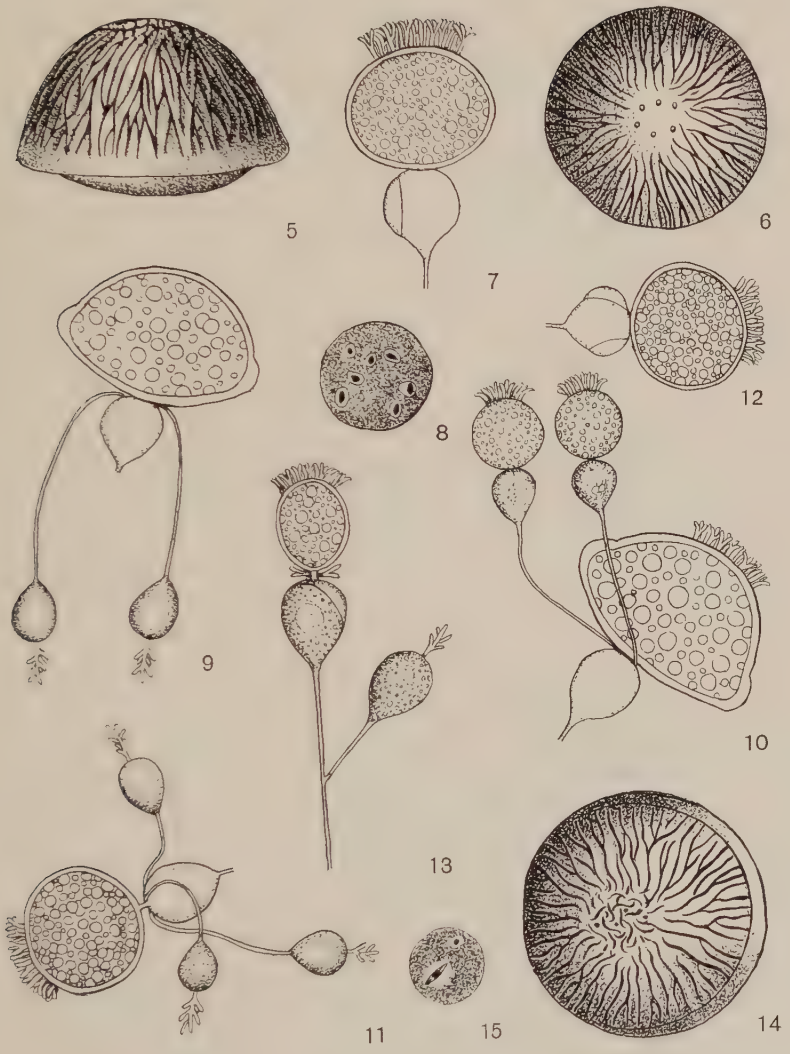
UROPHLYCTIS POTTERI



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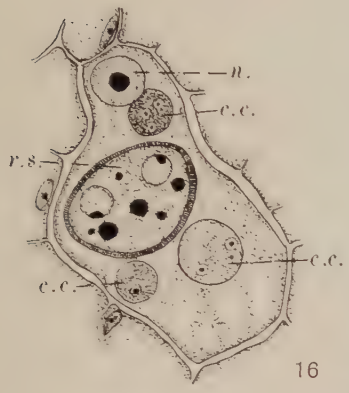

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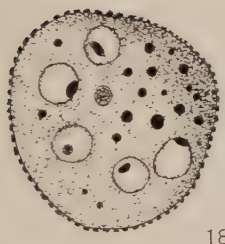

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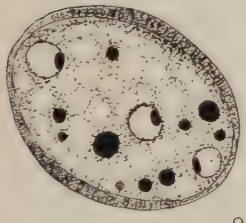

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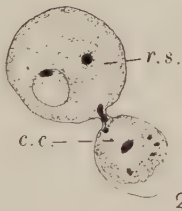

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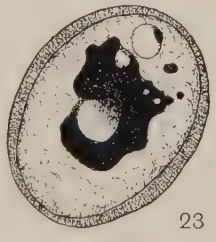

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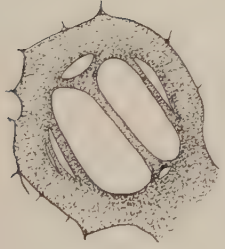

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UROPHLYCTIS POTTERI

Fig. 5. Mature resting sporangium, seen from the side. × 700.
Fig. 6. Immature resting sporangium, dorsal view. × 700.
Fig. 7. Young resting sporangium with empty collecting cell. × 700.
Fig. 8. Collecting cell in transverse section. × 700.
Figs. 9–13. Immature resting sporangia in various stages of development, showing origin of new collecting cells and sporangia. × 700.
Fig. 14. Mature resting sporangium, from above. × 700.
Fig. 15. Early stage of collecting cell, containing a nucleus apparently undergoing karyokinesis. × 1000.
Figs. 16 and 17. Sections of cavities in galls, showing collecting cells (*c.c.*) and resting sporangia (*r.s.*) in various stages of development. *n.*, *n.* are enlarged nuclei of host-cells. × 500.
Figs. 18, 19 and 21. Sections of resting sporangia, showing stages in development. × 800.
Fig. 20. Median vertical section of mature resting sporangium, containing large central mass of chromatin. *en.*, endospore; *ex.*, exospore. × 700.
Fig. 22. Young resting sporangium (*r.s.*) receiving contents of collecting cell (*c.c.*). A nucleus is seen in the narrow passage connecting the two. × 800.
Fig. 23. Maturing resting sporangium with large central mass of chromatin. × 800.
Figs. 24 and 25. Perforations in cell-walls between adjoining cavities. × 800.
Fig. 26. Section of immature collecting cell. × 800.

A NOTE ON BOTRYODIPLODIA SP. ON CHOISYA TERNATA IN ENGLAND

(With 4 Text-figs.)

By R. C. Woodward,

School of Rural Economy, Oxford.

A PYCNIDIAL fungus causing a dying back of the extremities of twigs of *Choisya ternata* was investigated in the spring of 1923. The organism isolated from dead branches of this shrub kindly sent by Mr W. J. Dowson of Wisley and also from specimens in the Cambridge Botanic Gardens proved to be a species of *Botryodiplodia*, a genus rare in temperate regions but widespread both as a saprophyte and as a parasite in the tropics.

The fungus, of which a single spore isolation was made, produced a good vegetative growth on potato agar similar in character to that of *Botryodiplodia Theobromae* Pat. Sterilised twigs of *Choisya ternata* in culture tubes served as a very satisfactory medium; the fungus penetrated all the tissues and eventually formed pycnidia in the bark. After some time soft cushion-like masses of vegetative growth of a sooty appearance were formed. The brown, thick-walled, uniseptate spores germinated rapidly and vigorously in water, they retained their vitality over long periods and resisted desiccation. Spores, in a sterilised twig-culture, germinated freely when immersed in water after remaining three years in a dry condition.

A number of inoculations were carried out on a healthy shrub of *Choisya ternata* in the Botanic Gardens, Cambridge. A pure culture of mycelium was used as the inoculum; inoculations were made with a sterile scalpel, and uninoculated, but otherwise similarly treated branches, served as controls; all the controls remained uninfected. Ten out of twelve inoculations proved successful.

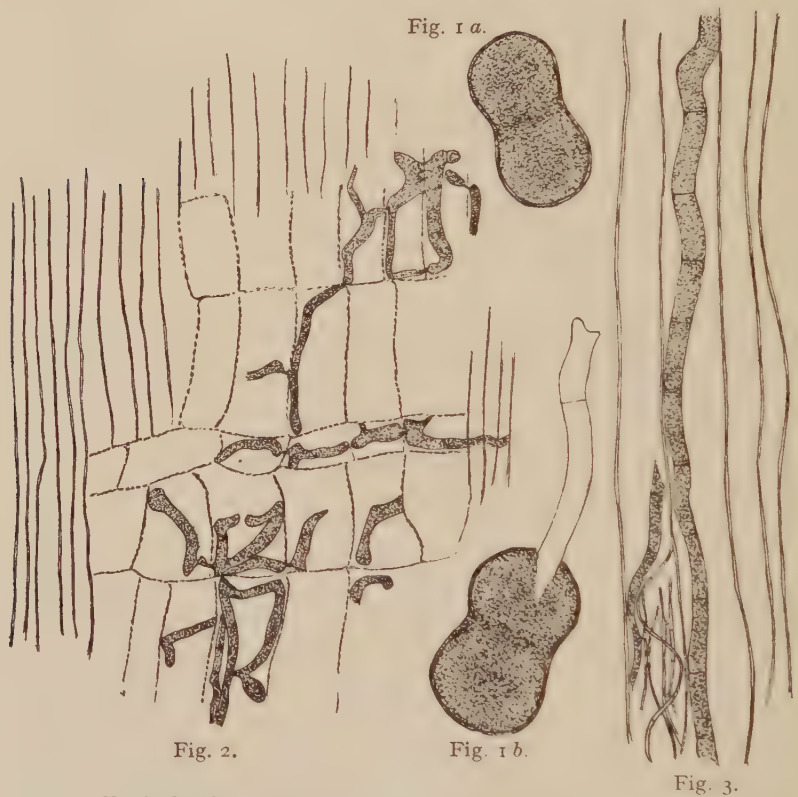

Fig. 1 *a*. Typical uniseptate spore.
Fig. 1 *b*. Germinating spore with hyaline germ-tube.
Fig. 2. Characteristic appearance of mycelium in medullary ray. Note the penetration of cell-walls by way of pits.
Fig. 3. Large, dark-brown hyphae in the vessels accompanied by smaller hyaline hyphae.

The fungus is remarkable for its rapidity of attack; inoculations made in May on living healthy branches killed them outright within a month.

The dead branches presented a great contrast to the healthy ones owing to the brown withered leaves which persisted on

them. In a successful inoculation a darkened area usually occurred near the callus which had formed over the inoculation wound. On splitting the branch longitudinally the woody tissue in the vicinity of the wound was seen to be discoloured; the pith was brown, the discoloration extending further in this region than in the wood. The penetration of cell walls takes place by way of the pits. The hyphae frequently anastomose. The pith and wood are darkened by the more mature hyphae, which are dark brown in colour. The darkened tissues, however, do not indicate the full area of invasion; thin, hyaline hyphae may be seen extending further in the vessels and medullary rays. Whereas no staining is required to show the distribution of the older hyphae, delicate staining is necessary to differentiate the very fine hyaline hyphae which initiate and extend the invasion of the tissues. The death of the infected branch is probably caused by the cutting off of the water supply by hyphae in the vessels. Fructifications were formed in the dead bark; they were frequently close together, forming a continuous slightly projecting mass of minute pinnacles.

In the absence of host plants upon which inoculations might be carried out to establish the species the specific name *Botryodiplodia Theobromae* Pat. may be only provisionally accepted.

The fungus has received a large number of different names, among which are *Macrophoma vestita*, *Diplodia cacaoicola*, *Lasiodiplodia Theobromae*, and *Diplodia rapax*. Petch (1) gives the following host plants: cacao, sugar cane, *Albizzia Moluccana*, papaw, mango, castilloa, *Hevea brasiliensis*, dadap, *Ficus elastica*, tea, and coconut. It has recently been reported as causing a gumming degeneration of tissues of the tobacco plant in Brazil (2) and as an apple root disease in Kenya (3).

My thanks are due to Mr F. T. Brooks, who suggested an investigation of this fungus and who pointed out its similarity to *Botryodiplodia Theobromae* as seen in the tropics. This work was done in the Cambridge University Botany School.

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ON TWO SPECIES OF TOLYPOSPORIUM WORONIN RECORDED ON CULTIVATED SORGHUM

By E. W. Mason, M.A., M.Sc.,

Imperial Bureau of Mycology.

I. *Tolyposporium Ehrenbergii* (Kühn) Pat. = *Tolyposporium filiferum* Busse.

Last year (1925) Zaprometoff published a book on the diseases of plants in Middle Asia, and in it gave an account of *Sorosporium Ehrenbergii* Kühn on sorghum (*Andropogon Sorghum*). On request, he kindly supplied specimens of the fungus on *Andropogon Sorghum contractus* Kcke., collected by A. Pospeloff in the Bokhara region in August, 1925. They were recognised, on receipt, as the same smut which is recorded in Africa and India as *Tolyposporium filiferum* Busse. As Kühn's name antedates Busse's by many years, it became necessary to determine whether Zaprometoff's determination could be substantiated.

The history of *Sorosporium Ehrenbergii* is as follows. Schweinfurth collected specimens at Cairo, Egypt, in 1876, and forwarded them to Thümen, who issued them in his *Mycotheca Universalis*, No. 725, as *Ustilago Reiliana* Kühn f. *Sorghi-cernui* on *Sorghum cernuum* Willd. [*Andropogon Sorghum* Brot.]. Kühn examined a specimen and finding that it was not his species, and not even congeneric with it, named it *Sorosporium Ehrenbergii*.

Thümen's *Mycotheca Universalis*, No. 725 in Herb. Kew, is identical with Zaprometoff's material, and there can be no doubt that it is Kühn's species of which a full account is given in *Hedwigia*, xvii (1878), pp. 12–14. His species is differentiated from the other smuts on sorghum by the aggregation of its spores into spore balls, as in *Sorosporium bullatum* Schroet. [= *Tolyposporium bullatum* Schroet. 1887] on *Echinochloa crus-galli*. Unlike the latter species, however, the individual spore sacs are remarkably long, 8 to 13 mm. long and 3 to 5 mm. broad. The usual diameter of the spores is 12·4 μ.

As Thümen's No. 725 in Herb. Kew shows just these characters, it must be taken that it is genuine *Sorosporium Ehrenbergii* Kühn, and that Zaprometoff's determination is correct. Further, Busse's description and figures of his *T. filiferum* leave no doubt that he was dealing with the same fungus. Sydow's *Fungi Exotici Exsiccati*, No. 351, in Herb. Imp. Bur.

Myc., issued as *Tolyposporium filiferum* collected by W. McRae, Government Farm, Coimbatore, Madras Presidency, India, is identical with the Egyptian and Middle Asian material. The specific name *filiferum* must, therefore, be abandoned. On the other hand, the presence of the persistent spore-balls places the fungus in the genus *Tolyposporium*. The first time I have found it definitely listed in this genus is in 1903 by Patouillard. The correct citation therefore appears to be

Tolyposporium Ehrenbergii (Kühn) Pat. in Bull. Soc. Mycol. XIX (1903), p. 254. Synon. *Sorosporium Ehrenbergii* Kühn in Die Brandenformen der Sorghum-arten. Ein Beitrag zur Geographie der Pflanzenkrankheiten. Mitt. Ver. Erdkunde Halle (1887), p. 87 (not seen); and Hedwigia, XVII (1888), p. 13. *Tolyposporium filiferum* Busse in Untersuchungen über die Krankheiten der Sorghum-hirse. Arb. K. Biol. Abt. für Land-Forstwirtsch. IV (1904), 4, p. 384.

The fungus has been recorded as *T. filiferum* in Tanganyika territory (formerly German East Africa) by Busse (*loc. cit.*); in British India by Sydow and Butler (1906), by Butler (1918) and by Kulkarni (1918); in Egypt, the type locality of *S. Ehrenbergii*, by Briton-Jones (1922), who states that it is there the commonest smut of sorghum. In 1921, Thomas baldly recorded the "long smut" of sorghum for Mesopotamia; long smut is a popular name for this fungus, but no description is given. Under the specific name of *Ehrenbergii*, it is recorded from Egypt by Kühn (*loc. cit.*), from Tunis by Patouillard (*loc. cit.*) and now from Central Asia by Zaprometoff (1925).

The smut can easily be recognised by the following characters:

(1) the long spore sacs, each confined to one spikelet of the host;

(2) the presence of a number (up to ten) of brown threads in each spore sac, in place of a central columella;

(3) the aggregation of the spores into spore balls, which do not fall to pieces under the coverslip;

(4) the angled spores with a mean diameter of about 12 μ.

It is satisfactory to note that the fourth and last smut of sorghum known to Kühn has been recognised again, in view of Reed's (1925) statement that while Kühn's description corresponds closely with *Sorosporium Reilianum*, the specimen of Thümen's *Mycotheca Universalis*, No. 725, at the New York Botanical Gardens, cannot be distinguished from *Sphacelotheca Sorghi*. Kühn's real species is clearly distinct from either of these.

II. *Tolyposporium Volkensii* Henn.

This species on cultivated *Sorghum* sp. was founded by Hennings in Engler, *Die Pflanzenwelt Ost-Afrikas und der Nachbargebiete*, Theil C, p. 49 (1895), on a collection made by

Volkens at Kilimandscharo, East Africa. The species does not appear to have been recognised again, and there has been some speculation among tropical mycologists as to the identity of this species. A collection in Herb. Kew, labelled *Tolyposporium Volkensii* Henn., Flora des Kilimandscharo No. 296, Marangu, May 1893, leg. G. Volkens, had been referred there to *Cerebella Andropogonis* Ces.

As the authenticity of this material was doubtful, Hennings's type specimen was obtained through the courtesy of Professor Diels, Director of the Botanical Museum, Fredrich-Wilhelm's Universität, Berlin-Dahlem, and the material at Kew proved to be genuine *Tolyposporium Volkensii* Henn. It is also identical with a collection received from J. McDonald, Kenya, and which was referred at this Bureau to *Cerebella Sorghi-vulgaris* Subram. (*Journ. and Proc. Asiatic Soc. of Bengal* (N.S.), XVII (1921), 206). Subramaniam has kindly confirmed this latter determination.

The species is a good *Cerebella*, and easily to be distinguished from a smut. Under a lens, each black stroma can be seen to be folded like the convolutions on the surface of the brain, and a section shows that the stroma is covered with a palisade of conidiophores, each of which carries at its apex a conidium with septa in three planes. The correct specific names to apply to the *Cerebella* spp. on cultivated sorghums, if there are more than one, is not yet quite clear. Mr McDonald is now studying them in culture, and it is hoped that this will lead to more definite conclusions. The present note is only intended to record that *Tolyposporium Volkensii* Henn. (1895) recorded on sorghum from East Africa is not a smut but a species of *Cerebella*, and that it is identical with *Cerebella Sorghi-vulgaris* Subram. (1921), recorded on the same host from India and the Philippines.

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Lepiota gracilenta (Krombh.) Fr.

E. A. & V. Rea del.

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79. Cory, Mr F. M., Botanical Department, The University, Bristol. (1926.)
80. Cotton, Mr Arthur D., F.L.S., Keeper, Herbarium, Royal Botanic Gardens, Kew, Surrey. (1902.)
81. Crow, Mr W. B., M.Sc., F.L.S., Botanical Department, University College, Cardiff. (1921.)
82. Cunningham, Mr G. H., Biological Laboratory, 71, Fairlie Terrace, Kilburn, Wellington, New Zealand. (1922.)
83. Curtis, Miss Kathleen M., M.A., D.Sc., D.I.C., F.L.S., Mycologist, Biological Department, Cawthron Institute of Scientific Research, Nelson, New Zealand. (1917.)
84. Cutting, Mr E. M., M.A., F.L.S., Botanical Department, University College, Gower Street, London, W.C. 1. (1920.)
85. Darbishire, Professor O. V., B.A., Ph.D., F.L.S., The University, Bristol. (1913.)

86. Das, Mr Kedarnath, C.I.E., M.D., Principal, Carmichael Medical College, 1, Belgachia Road, Calcutta, India. (1922.)
87. Davies, Mr D. W., B.Sc., Advisor in Mycology, Agricultural Buildings, University College of Wales, Aberystwyth. (1923.)
88. Davis, Mr J. Jefferson, B.S., M.D., University of Wisconsin, Madison, Wis., U.S.A. (1921.)
89. Day, Mr E. Metcalfe, Rowan Cottage, Minchinhampton, Glos. (1921.)
90. Deighton, Mr F. C., B.A., Mycologist, Department of Agriculture, Freetown, Sierra Leone, West Africa. (1925.)
91. Dickinson, Mr S., 3, The Warren, Lillington, Leamington Spa. (1921.)
92. Dickson, Professor B. T., B.A., Ph.D., Macdonald College, St Anne de Bellevue, Quebec, Canada. (1923.)
93. Dowson, Mr W. J., M.A., F.L.S., Royal Horticultural Society's Gardens, Wisley, Ripley, Surrey. (1920.)
94. Doyle, Professor J., M.Sc., University College, Dublin. (1925.)
95. Duke, Miss M. M., B.Sc., Herbarium, Royal Botanic Gardens, Kew, Surrey. (1924.)
96. Edwards, Mr W. H., Curator, The Museum, Birmingham. (1896.)
97. Elliot, Rev. E. A., Dunstall Vicarage, Burton-on-Trent. (1923.)
98. Elliott, Mr W. T., D.D.S., L.D.S., F.L.S., F.Z.S., Arden Grange, Tanworth-in-Arden, Warwickshire. (1913.)
99. Elliott, Mrs J. S. Bayliss, D.Sc. (B'ham), B.Sc. (Lond.), Arden Grange, Tanworth-in-Arden, Warwickshire. (1911.)
100. Ellis, Mr David, D.Sc., Ph.D., F.R.S.E., Royal Technical College. (1923.)
101. Ellis, Mr E. H., British Museum (Nat. Hist.), Cromwell Road, London, S.W. 7. (1924.)
102. Engledow, Mr F. L., M.A., School of Agriculture, Cambridge (1922.)
103. Essex Field Club, c/o Mr Percy Thompson, F.L.S., Essex Museum of Natural History, Romford Road, Stratford, London, E. 15. (1919.)
104. Exeter, Biological Department, University College of the South-West of England. (1926.)
105. Eyre, Miss J. C., Maitlands Cottage, Ipplepen, Newton Abbot, Devon. (1915.)
106. Fenton, Mr E. W., M.A., B.Sc., F.L.S., Botanical Department, Seale Hayne Agricultural College, Newton Abbot, Devon. (1920.)
107. Finlayson, Mr Raymond A., F.L.S., Official Seed Testing Station, Huntingdon Road, Cambridge. (1910.)
108. Fry, Miss E. J., "Hazelhurst," Pear Tree Avenue, Bitterne, Southampton. (1923.)

109. Gadd, Mr C. H., D.Sc., Ceylon Tea Research Association, c/o Royal Botanic Gardens, Peradeniya, Ceylon. (1921.)
110. Gardner, Capt. Frederic, c/o Lloyd's Bank, Jersey, C.I. (1898.)
111. Garside, Mr S., M.Sc., F.L.S., Botanical Department, Bedford College, Regent's Park, London, N.W. 1. (1922.)
112. Gates, Professor R. R., B.Sc., Ph.D., F.L.S., King's College, Strand, London, W.C. (1921.)
113. Gilbert, M. E., Docteur en Pharmacie, 6, Rue de Laos, Paris (15e), France. (1924.)
114. Gilbert, Dr E. M., Botanical Department, University of Wisconsin, Madison, Wis., U.S.A. (1922.)
115. Gilchrist, Miss Grace G., B.Sc., Botanical Department, The University, Bristol. (1921.)
116. Gorman, Mr M. J., A.R.C.Sc.I., College of Science, Upper Merrion Street, Dublin. (1925.)
117. Gossling, Mrs W. L., 20, Carlton Hill, London, N.W. 8. (1922.)
118. Gough, Mr G. C., B.Sc., A.R.C.S., Ministry of Agriculture, Birmingham. (1923.)
119. Gould, Mr F. G., Elmhurst, Church Hill, Loughton, Essex. (1918.)
120. Gould, Mr N. G., Royal Horticultural Society's Gardens, Wisley, Ripley, Surrey. (1922.)
121. Green, Col. C. Theodore, A.M.S., M.R.C.S., L.R.C.P., F.L.S., 31, Shrewsbury Road, Birkenhead. (1901.)
122. Green, Miss E., 9, Brunswick Square, London, W.C. (1925.)
123. Green, Mr E. Ernest, F.Z.S., F.E.S., Way's End, Camberley, Surrey. (1917.)
124. Grey, Mrs O., F.Z.S., 90, Charing Cross Road, London, W.C. 2. (1926.)
125. Grinling, Mr C. H., B.A., 71, Rectory Place, Woolwich, London, S.E. 18. (1913.)
126. Gunter, Mr Thomas J., 4, Alexandra Road, London, N. 4. (1926.)
127. Gwynne-Vaughan, Professor Dame Helen, D.Sc., LL.D., F.L.S., 93, Bedford Court Mansions, London, W.C. 1. (1906.)
128. Haas, Mr P., D.Sc., Ph.D., F.C.S., University College, Gower Street, London, W.C. 1. (1921.)
129. Hadden, Mr Norman G., Underway, West Porlock, Somerset. (1911.)
130. Hanna, Mr W. F., M.Sc., University of Alberta, Edmonton, Alberta, Canada. (1925.)
131. Hansford, Mr C. G., M.A., Mycologist, Department of Agriculture, Kampala, Uganda. (1921.)
132. Harvard University, The Library, Cambridge, Mass., U.S.A. (1923.)
133. Hare, Mr J. G., Molteno Institute of Parasitology, Cambridge. (1924.)

134. Harris, Mr R. V., B.Sc., Horticultural Research Station, East Malling, Kent. (1924.)
135. Harvey, Mrs Cecily D., Barwick-in-Elmet Rectory, near Leeds. (1910.)
136. Hasluck, Miss I. E., Green Hill Park, New Barnet, Herts. (1922.)
137. Hastings, Mr Somerville, M.S., F.R.C.S., 43, Devonshire Street, Portland Place, London, W. 1. (1913.)
138. Hemmi, Dr Takewo, Phytopathological Institute, Department of Agriculture, Kyoto Imperial University, Kyoto, Japan. (1923.)
139. Hildyard, Mr F. W., 14, Lambridge, Bath. (1913.)
140. Hiley, Mr Wilfred E., M.A., F.L.S., Research Institute, School of Forestry, Oxford. (1913.)
141. Hoare, Mr A. H., 111, Blenheim Gardens, Wallington, Surrey. (1922.)
142. Hoggan, Miss I. A., Newnham College, Cambridge. (1923.)
143. Holden, Dr H. S., F.L.S., Botanical Department, University College, Nottingham. (1923.)
144. Honolulu, The Library, Experiment Station, S.P.A., Box 411, Hawaii. (1920.)
145. Horne, Mr A. S., D.Sc., F.L.S., F.G.S., Botanical Department, Imperial College of Science, South Kensington, London, S.W. 7. (1921.)
146. Howard, Mr H. J., F.R.M.S., "Lingfield," 6, College Road, Norwich. (1918.)
147. Hughes, Mr G. C., Chesterton, Bicester, Oxon. (1898.)
148. Humphrey, Mr C. J., Mycologist and Plant Pathologist, Bureau of Science, Manila, Philippine Islands. (1921.)
149. Hunter, Mr C., M.Sc., Botanical Department, The University, Bristol. (1921.)
150. Hurrell, Mr H. E., 25, Regent Street, Great Yarmouth. (1921.)
151. Hyde, Mr H. A., M.A., F.L.S., National Museum of Wales, Cardiff. (1924.)
152. Imperial College of Tropical Agriculture, Trinidad, B.W.I. (1921.)
153. Iowa, The Library, State University of Iowa, Iowa City, U.S.A. (1923.)
154. Issatchenko, Professor B. L., Directeur du Jardin Botanique, Petrograd. (1923.)
155. Jaczewski, Professor Arthur de, Director, Institute of Mycology and Phytopathology, Perspective Anglaise 29, Petrograd, Russia. (1922.)
156. John Innes Horticultural Institute, Mostyn Road, Merton, Surrey. (1924.)
157. Johnson, Mr J. W. Haigh, M.Sc., F.I.C., F.L.S., Walton, near Wakefield (1919.)

158. Johnstone, Mr R. B., 3, Oswald Gardens, Scotstounhill, Glasgow. (1908.)
159. Jones, Mr G. H., M.A., Department of Agriculture, Ibadan, South Nigeria. (1922.)
160. Jones, Mr Robert Fowler, Trinity House, Denton Road, Ilkley, Yorks. (1918.)
161. Jørstad, Mr Ivar, Statsmykolog, Botanisk Museum, Christiania, Norway. (1923.)
162. Kavina, Professor Dr Karel, Professor of Botany, Havlickovy-sady 58, Praha-Kral. Vinohrady (Prague), Czechoslovakia. (1926.)
163. Keef, Miss Phoebe, Mortimer Lodge, Wimbledon Park, London, S.W. 17. (1921.)
164. Keilin, Dr D., Molteno Institute of Parasitology, Cambridge. (1922.)
165. Keissler, Dr Karl, Direktor d. Botanischen Abteilung, Naturhistorisches Museum, Burgring 7, Wien 1/1, Austria. (1924.)
166. Kelly, Dr Howard A., 1418, Eutaw Place, Baltimore, Md., U.S.A. (1921.)
167. Kendall, Miss Olwen, 9, Lordship Park, Stoke Newington, London, N. 16. (1921.)
168. Kew, The Library, Royal Botanic Gardens. (1921.)
169. Kidd, Mrs Franklin, B.A., The Botany School, Cambridge. (1919.)
170. Kirby, Mr E. E., B.A., Grafton House, Oxford Street, Norwich. (1924.)
171. Klika, Mr Bohumil, Hálkova 37, Prague, Vrsovice 553, Czechoslovakia. (1926.)
172. Knight, Mr H. H., M.A., The Lodge, All Saints' Villas, Cheltenham. (1914.)
173. Knowles, Miss M. C., M.R.I.A., Natural History Museum, Dublin. (1925.)
174. Krieger, Mr L. C. C., 2114, N. Calvert Street, Baltimore, Md., U.S.A. (1921.)
175. Kulkarni, Mr G. S., M.Ag., Assistant Professor of Mycology, Agricultural College, Poona, India. (1922.)
176. Lampitt, Mr L. H., D.Sc., F.I.C., Thornlea, Mount Park, Harrow, Middlesex. (1925.)
177. Latter, Miss Joan, Botanical Department, King's College, Strand, London, W.C. 2. (1923.)
178. Leicester, The Museum, City of Leicester. (1923.)
179. Lewis, Professor F. J., D.Sc., University of Alberta, Edmonton, Alberta, Canada. (1924.)
180. Line, Mr James, M.A., School of Agriculture, Cambridge. (1921.)

181. Linnean Society, The, Burlington House, Piccadilly, London, W. 1. (1919.)
182. Lloyd, Mr C. G., The Lloyd Library and Museum, 224, West Court Street, Cincinnati, Ohio, U.S.A. (1907.)
183. Lowndes, Mr A. G., M.A., Marlborough College, Marlborough, Wilts. (1922.)
184. MacCallum, Mrs B. D., M.A., D.Sc., F.L.S., c/o Professor MacCallum, Department of Pathology, University of Melbourne, Australia. (1921.)
185. Mackenzie, Miss A. D., Research Station, East Malling, Kent. (1921.)
186. Mackenzie, Mr D., . (1900.)
187. Madras University Library, Madras, South India. (1925.)
188. Maire, M. René, D.Sc., Professeur à la Faculté des Sciences de l'Université, Algers, Algeria, N. Africa. (1907.)
189. Maitland, Mr T. D., M.B.E., Government Botanist, Department of Agriculture, Kampala, Uganda. (1916.)
190. Maltby, Mr G. C., 14, Northwick Road, Evesham. (1923.)
191. Marmont, Mr Basil P., Windsoredge House, Inchbrook, near Woodchester, Glos. (1908.)
192. Marriott, Mr St John, 37, Owenite Street, Abbey Wood, London, S.E. 2. (1920.)
193. Marsh, Mr R. W., M.A., Research Station, Long Ashton, Bristol. (1923.)
194. Mason, Mr E. W., M.A., M.Sc., Imperial Bureau of Mycology, 17, Kew Green, Kew, Surrey. (1921.)
195. Mason, Mrs E. W., Suffield House, Paradise Road, Richmond, Surrey. (1922.)
196. Mason, Mr F. A., F.R.M.S., M.S.P.A., 29, Frankland Terrace, Leeds. (1912.)
197. Mason, Mr F. R., Assistant Mycologist, Department of Agriculture, Kuala Lumpur, Federated Malay States. (1921.)
198. Matthews, Mr J. R., M.A., F.L.S., Royal Botanic Gardens, Edinburgh. (1921.)
199. Mehta, Professor K. C., Ph.D., Department of Biology, Agra College, Agra, U.P., India. (1921.)
200. Melvill, Mr J. Cosmo, M.A., D.Sc., F.L.S., Meole Brace Hall, Shrewsbury. (1922.)
201. Menzies, Mr James, 117, Scott Street, Perth. (1917.)
202. Meulenhoff, Dr J. S., President, Dutch Mycological Society, Diezerstraat, Zwolle, Holland. (1921.)
203. Millard, Mr W. A., B.Sc., The Agriculture Department, The University, Leeds. (1924.)
204. Missouri, The Botanical Garden, St Louis, Mo., U.S.A. (1902.)
205. Miyabe, Dr Kingo, Professor of Botany, Hokkaido Imperial University, Sapporo, Japan. (1919.)

206. Montague, Mrs A., Penton, Crediton, N. Devon. (1898.)
207. Moore, Miss E. S., Ph.D., c/o the Secretary, Department of Agriculture, P.O. Box 994, Pretoria, South Africa. (1923.)
208. Moore, Mr W. C., M.A., Ministry of Agriculture, Pathological Laboratory, Milton Road, Harpenden, Herts. (1922.)
209. Morris, Mr L. E., The Shirley Institute, Didsbury, Manchester. (1924.)
210. Moss, Professor C. E., The University, P.O. Box 1176, Johannesburg, South Africa. (1923.)
211. Mottram, Miss W. E., B.Sc., Horton Lane, Epsom, Surrey. (1925.)
212. Mundkur, Mr B. B., M.A., Cotton Research Laboratory, Government Farm, Dharwar, India. (1924.)
213. Murphy, Mr P. A., Sc.D., A.R.C.Sc.I., M.R.I.A., Plant Diseases Division, College of Science, Upper Merrion Street, Dublin. (1924.)
214. Murray, Mr G. H., F.E.S., Papuan Government Service, Port Moresby, Papua, British New Guinea. (1921.)
215. Murrell, Major Percy J., O.B.E., F.R.M.S., "Littlecroft," Orpington, Kent. (1923.)
216. Muskett, Mr A. E., B.Sc., A.R.C.S., Queen's University, Belfast, North Ireland. (1923.)
217. McDonald, Mr J., B.Sc., Mycologist, Department of Agriculture, Box 323, Nairobi, Kenya Colony, East Africa. (1923.)
218. McDougall, Professor W. B., University of Illinois, Urbana, Ill., U.S.A. (1921.)
219. McFarland, Dr Frank T., Department of Botany, University of Kentucky, Lexington, Ky., U.S.A. (1924.)
220. McLean, Professor R. C., M.A., D.Sc., F.L.S., Botany School, University College, Cardiff. (1922.)
221. McLennan, Dr Ethel I., Botanical Department, Melbourne University, Carlton, Victoria, Australia. (1926.)
222. Nagpur, The Mycologist to the Government, C.P., India. (1924.)
223. Nattrass, Mr R. M., B.Sc. (Agric.), Research Station, Long Ashton, Bristol. (1925.)
224. Nebraska, The Library, University of, Lincoln, Nebr., U.S.A. (1924.)
225. Nederlandsche Mycologische Vereeniging, c/o H. A. A. van der Lek, Zoomweg 10, Wageningen, Holland. (1920.)
226. Newcastle-upon-Tyne, Literary and Philosophical Society, c/o Mr H. Richardson, Librarian. (1902.)
227. Newton, Mr W. C. F., B.Sc., John Innes Horticultural Institute, Mostyn Road, Merton, Surrey. (1922.)
228. New York Botanical Garden, Bronx Park, New York, U.S.A. (1904.)

229. Nicholson, Mr W. E., F.L.S., 50, St Anne's Crescent, Lewes. (1913.)
230. Noel, Miss E. F., F.L.S., 37, Moscow Court, Queen's Road, London, W. 2. (1913.)
231. North Carolina, Library, University of, Chapel Hill, North Carolina, U.S.A. (1920.)
232. Nursery and Market Garden Industries' Development Society, Ltd., Experimental and Research Station, Cheshunt, Herts. (1922.)
233. O'Connor, Mr P., B.Sc., A.R.C.Sc.I., College of Science, Upper Merrion Street, Dublin. (1925.)
234. Ogilvie, Mr L., M.A., M.Sc., Department of Agriculture, Agricultural Station, Paget East, Bermuda. (1922.)
235. Oke, Mr Alfred William, B.A., F.G.S., F.L.S., 32, Denmark Road, Hove, Sussex. (1908.)
236. Oldham, Mr C. H., Ivy Dene, Chandler's Ford, Southampton. (1923.)
237. Ontario Agricultural College, Library, Guelph, Ontario, Canada. (1920.)
238. Osborn, Professor T. G. B., M.Sc., Adelaide University, Adelaide, South Australia. (1910.)
239. Ottawa, Ontario, Canada, The Library, Geological Survey. (1926.)
240. Overeem, Dr C. van, Buitenzorg, Java. (1920.)
241. Page, Miss W. M., B.Sc., 19, Ledam Buildings, Bourne Estate, Holborn, London, E.C. 1. (1921.)
242. Pan, Mr T. C., M.B., Ch.B., Avondale, Lenzie, Glasgow. (1925.)
243. Parke Davis & Co., Librarian, Research Department, Detroit, Michigan, U.S.A. (1920.)
244. Paul, The Very Rev. David, D.D., LL.D., 53, Fountainhall Road, Edinburgh. (1899.)
245. Paulson, Mr Robert, F.L.S., F.R.M.S., Glenroy, Cecil Park, Pinner, Middlesex. (1918.)
246. Peacock, Dr H. G., The Lawn, Torquay. (1896.)
247. Pearson, Mr Arthur A., F.L.S., 59, Southwark Street, London, S.E. 1. (1911.)
248. Peklo, Dr Jaroslav, Professor of Applied Botany, Bohemian Technical University, Charles Square, Prague II, Czecho-Slovakia. (1924.)
249. Perthshire Society of Natural Science, c/o Mr James Winter (Hon. Treasurer), 35, George Street, Perth. (1919.)
250. Petch, Mr T., B.A., B.Sc., Tea Research Institute, Nuwara Eliya, Ceylon. (1911.)
251. Pethybridge, Mr G. H., Ph.D., B.Sc., Ministry of Agriculture, Pathological Laboratory, Milton Road, Harpenden, Herts. (1919.)

252. Philadelphia, The Academy of Natural Sciences of Philadelphia, Logan Square, Phila., U.S.A. (1925.)
253. Phillips, Dr H. H., 6, St John's Road, Penge, London, S.E. 10. (1923.)
254. Phillips, Mr F. J., Research Officer, Forest Research Station, Deepwalls, Knysna, South Africa. (1921.)
255. Ping, Mr A. Wentworth, M.A., "St Olave's," Clifton, York. (1926.)
256. Plowright, Mr C. T. M., B.A., M.B., King Street, King's Lynn. (1901.)
257. Potter, Rev. M. C., Sc.D., M.A., F.L.S., Corley Cottage, York Avenue, New Milton, Hants. (1896.)
258. Povah, Professor A. H., 9, Fisk Hall, Northwestern University, Evanston, Ill., U.S.A. (1924.)
259. Preston, Mr N. C., B.Sc., Harper Adams Agricultural College, Newport, Salop. (1920.)
260. Pretoria, South Africa, The Chief, Division of Botany (91403), Department of Agriculture. (1922.)
261. Priestley, Professor J. H., D.S.O., B.Sc., F.L.S., Botanical Department, The University, Leeds. (1912.)
262. Priestley, Mrs Marion E., 2, Balmoral Terrace, Shaw Lane, Headingley, Leeds. (1919.)
263. Pusa, Imperial Mycologist, Imperial Agricultural Research Institute, Pusa, Bihar, India. (1921.)
264. Ramsbottom, Mr J., O.B.E., M.A., F.L.S., British Museum, Cromwell Road, South Kensington, London, S.W. 7. (1910.)
265. Rayner, Mr J. F., Swaythling, Southampton. (1902.)
266. Rayner, Miss M. Cheveley, D.Sc., Bedford College for Women, Regent's Park, London, N.W. 1. (1921.)
267. Rea, Mrs E. A., 6, Barbourne Terrace, Worcester. (1896.)
268. Rea, Miss M. W., M.Sc., Salem House, Sydenham, Belfast, Ireland. (1920.)
269. Rea, Miss Violet, 6, Barbourne Terrace, Worcester. (1921.)
270. Reichert, Dr Israel, Plant Pathologist, Palestine Zionist Executive Agricultural Experiment Station, Tel-Aviv, Palestine. (1924.)
271. Rhind, Mr Donald, B.Sc., Mycologist, Department of Agriculture, Agricultural College, Mandalay, Burma. (1922.)
272. Rhodes, Miss Mabel, Lister Institute, Chelsea Gardens, London, S.W. 1. (1921.)
273. Richards, Mr R. M., M.B.E., A.R.C.S., F.L.S., The Laboratory, Caledonia Estate, Province Wellesley, Straits Settlements. (1915.)
274. Roberts, Mrs A. W. Rymer, The End House, Fulbrook Road, Cambridge. (1920.)

275. Robinson, Wilfred, Department of Botany, Prof. of University College of Wales, Aberystwyth. (1923.)
276. Robson, Mr R., M.Sc., F.Z.S., Writtle, Chelmsford, Essex. (1914.)
277. Rolfe, Mr F. W., Colonial and Indian Collections, Imperial Institute, London, S.W. 7. (1923.)
278. Rolfe, Mr R. T., F.I.C., 76, Spenser Road, Bedford. (1924.)
279. Roper, Miss Ida M., F.L.S., 4, Woodfield Road, Redland, Bristol. (1921.)
280. Rothamsted Experimental Station, Department of Mycology, Harpenden, Herts. (1923.)
281. Rushton, Mr W., A.R.C.S., D.I.C., St Thomas's Hospital Medical School, Albert Embankment, London, S.E. 1. (1914.)
282. Rutgers College and State University of New Jersey, Library, New Brunswick, New Jersey, U.S.A. (1922.)
283. Ryan, Mr G. M., F.L.S., 35, Ladbroke Gardens, London, W. 11. (1923.)
284. St Paul, Minnesota, U.S.A., The Library, Department of Agriculture, University Farm. (1920.)
285. Salisbury, Mr E. J., D.Sc., F.L.S., Botanical Department, University College, Gower Street, London, W.C. 1. (1921.)
286. Salmon, Professor E. S., F.L.S., South-Eastern Agricultural College, Wye, Kent. (1922.)
287. Sampson, Miss K., B.Sc., University College, Aberystwyth, North Wales. (1920.)
288. Samuel, Mr Geoffrey, The University of Adelaide, South Australia. (1923.)
289. Sanderson, Mr A. R., F.L.S., Research Laboratory, Rubber Growers' Association, Petaling, Federated Malay States. (1921.)
290. Schinz, Professor Dr, Botanical Garden and Museum, Zurich, Switzerland. (1921.)
291. Science Library, South Kensington, London, S.W. 7. (1924.)
292. Scott, Mr W. W., 23, Wood Lane, Highgate, London, N. 6. (1922.)
293. Searle, Mr G. Odell, B.Sc. (Agric.), Research Botanist, Linen Industry Research Association, Glenmore House, Lambeg, Lisburn, Ireland. (1920.)
294. Sharpe, Mr C. J., Brambleside, Manor Road, Sidcup. (1905).
295. Sharples, Mr A., A.R.C.S., D.I.C., Mycologist, Department of Agriculture, Kuala Lumpur, Federated Malay States. (1924.)
296. Shaw, Mr F. J. F., D.Sc., A.R.C.S., F.L.S., Imperial Agricultural Research Institute, Pusa, Bihar, India. (1920.)

297. Small, Mr W., M.B.E., M.A., Ph.D., B.Sc., Mycologist, Royal Botanic Gardens, Peradeniya, Ceylon. (1915.)
298. Smith, Mr Alexander, The Botany School, Cambridge. (1924.)
299. Smith, Mr F. E. V., B.Sc., Ministry of Agriculture, Pathological Laboratory, Milton Road, Harpenden, Herts. (1922.)
300. Smith, Miss K. E., 64, Coton Road, Nuneaton. (1913.)
301. Smith, Mr Noel J. G., 9, Braidburn Crescent, Edinburgh. (1924.)
302. Smith, Miss S. Somerford, 6, Barbourne Terrace, Worcester. (1923.)
303. Solberg, Miss Louise, Kobbervik Drammen, Norway. (1923.)
304. South London Botanical Institute, Tulse Hill, Herne Hill, London, S.E. 24. (1921.)
305. Southwell, Mr Herbert, A.R.C.S., 158, Lincoln Road, Peterborough. (1923.)
306. Stakman, Professor E. C., University of Minnesota, Minneapolis, Minn., U.S.A. (1922.)
307. Stansfield, Miss O. P., M.Sc., Milton Mount College, Crawley, Sussex. (1922.)
308. Stationery Office, H.M., Superintendent of Publications, Book Dept., Westminster, S.W. 1. (2 subscriptions.) (1920.)
309. Stelfox, Mrs, "Mayfield," Clareville Road, Rathgar, Dublin. (1925.)
310. Stirrup, Mr H. H., M.Sc., Midland Agricultural College, Sutton Bonington, Loughborough. (1922.)
311. Storey, Mr H. H., B.A., Natal Herbarium, Durban, South Africa. (1922.)
312. Sutherland, Mr G. K., M.A., D.Sc., F.L.S., 110, Brackenbury Road, Moor Park, Preston. (1914.)
313. Swansea Field Naturalists' Society, c/o Mr Alan Stuart, M.Sc., University College, Singleton, Swansea, South Wales. (1924.)
314. Swanton, Mr E. W., A.L.S., Educational Museum, Haslemere, Surrey. (1899.)
315. Swedish Academy of Sciences, Royal, Stockholm, Sweden. (1919.)
316. Sydney, Australia, The Librarian, University of. (1922.)
317. Tabor, Mr R. J., B.Sc., F.L.S., Botanical Department, Imperial College of Science, South Kensington, London, S.W. 7. (1914.)
318. Tagg, Mr H. F., F.L.S., Royal Botanic Garden, Edinburgh. (1921.)
319. Tandy, Mr Geoffrey, Department of Botany, British Museum, Cromwell Road, South Kensington, London, S.W. 7. (1925.)
320. Taylor, Miss B. K., 98, Cheyne Walk, Chelsea, London, S.W. 3. (1910.)

321. Taylor, Mr G. Crosbie, "The Garden," 20, Tavistock Street, Covent Garden, London, W.C. 2. (1924.)
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337. Welsford, Miss E. J., Mycologist, Department of Agriculture, Zanzibar, East Africa. (1924.)
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339. Weston, Mr W. A. R. Dillon, B.A., School of Agriculture, Cambridge. (1923.)
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357. Wright, Mr E. Barton, B.Sc., Oakleigh, Godstone, Surrey. (1926.)

RULES.

Society's name and objects.

1. The Society shall be called "The British Mycological Society," and its objects shall be the study of Mycology in all its branches.

Members of Society.

2. The Society shall consist of Honorary Members, Foundation Members and Ordinary Members; the number of Honorary Members shall be limited to 20, and that of Foundation Members to 100*, but the number of Ordinary Members shall be unlimited.

Honorary Members.

3. Honorary Members shall be persons of pre-eminence in Mycology, or who have rendered special service to the Society.

Foundation Members.

4. Foundation Members shall be those Members or Societies who joined the Society previous to the limit of 100 Members having been attained*.

Officers.

5. The Officers of the society shall consist of a President, one or more Vice-Presidents, Treasurer, Secretaries, and Editor or Editors. They shall be elected annually at the Annual General Meeting of the Society.

Government of Society.

6. The government of the Society shall be vested in a Council, which shall consist of the President and other Officers for the time being, together with two or more other Members who shall be elected annually at the General Meeting, and one-half of whom shall retire each year and not be eligible for immediate re-election. The Members to retire shall be those who have been longest in office or, in case of equality, shall be determined by ballot. Ex-Presidents are *ex officio* Members of the Council.

Every Meeting of the Council shall be duly summoned by the Hon. Secretary by at least seven days' notice in writing to each Member of the Council.

* The limit of 100 Foundation Members was reached 22nd Oct. 1903.

Period of Office.

7. The Officers and Council shall hold office as from the 1st of January following their election.

Election of Members.

8. Honorary Members shall only be elected at a Meeting of the Society by a majority of the Members then present.

All Ordinary Members shall be proposed and seconded respectively by existing Members, who shall sign a certificate (see appendix) of recommendation, one at least of the proposers so certifying from personal knowledge. Every candidate for election shall sign an undertaking to abide by the Rules if elected (see appendix). They shall be elected by a majority of the Members present at any meeting of the Society or by the President and Officers of the Society.

Subscription.

9. All Ordinary Members and Societies shall pay an annual subscription of one pound, and Foundation Members five shillings, due on the 1st of January in each year. Honorary Members shall be exempt from any annual subscription.

Any Member wishing to retire from the Society shall give notice to the Hon. Secretary or Treasurer in writing before the 1st of December of the previous year.

Meetings.

10. The Society shall hold one or more Meetings annually, at a place and time determined by the Members at the preceding Annual General Meeting, or by the Council. The Annual General Meeting for the election of Officers and the transaction of other business shall coincide with the Autumn Foray.

Accounts.

11. At the Annual General Meeting of the Society in each year the Hon. Treasurer shall present duly audited accounts.

Alteration of Rules.

12. The Rules shall not be altered except by a two-thirds majority of the Members present at an Annual General Meeting. A printed copy shall be sent to every Member of the Society on election, and in the event of alteration to all Members.

APPENDIX.

Form of proposal for Ordinary Membership of the British Mycological Society.

.....

of .....

.....

being desirous of becoming an Ordinary Member of the British Mycological Society, we, the undersigned Members of the Society, certify that we consider h to be a desirable Member of the Society, and beg to recommend h for election.

Dated this day of 19

.....(From personal knowledge).

Certificate to be signed by the Candidate.

I hereby certify that I desire to become an Ordinary Member of the British Mycological Society and that I will abide by the Rules if elected.

.....

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